Exhibit 36

Risk factors for familial and sporadic ovarian cancer among French Canadians: A case-control study

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OBJECTIVE: The objective was to compare risk factors between familial and sporadic ovarian cancer by means of a case-control approach.

STUDY DESIGN: We conducted a case-control study among French Canadian women in Montreal during 1995-1996. One hundred seventy women 20 to 84 years old with histologically confirmed diagnoses of primary ovarian carcinomas or borderline tumors were interviewed concerning their reproductive, family, and medical histories. During the same period 170 randomly selected population control subjects, frequency-matched to the case patients according to age and ethnic group, were also interviewed. Unconditional logistic regression methods were used for data analysis.

RESULTS: The major factors influencing the risk of development of ovarian cancer were as follows: (1) family history of breast or ovarian cancer, (2) a late age at use of oral contraceptives (a protective effect), and (3) a late age at last childbirth (a protective effect for familial case patients only).

CONCLUSION: These factors had equally great impacts in familial and sporadic cases, implying that the underlying mechanisms of carcinogenesis in sporadic and familial ovarian cancer may be similar and that hereditary ovarian cancer may be preventable. (Am J Obstet Gynecol 1998;179:403-10.)

Key words: Case-control, epidemiology, family history, ovarian cancer, risk factors

Ovarian carcinoma is difficult to detect in its early stages and is resistant to therapy in later stages. Of the 2000 patients with new cases of ovarian cancer diagnosed every year in Canada, approximately 1350 (67.5%) will die of the disease.[1] The age-standardized incidence of ovarian cancer in Canada for 1996 was estimated to be 12 cases/100,000 at-risk population. The strongest risk factor for ovarian cancer is a positive family history of ovarian or breast cancer. Among common adult tumors, ovarian cancer has among the highest proportions attributable to susceptibility genes.[2] Other risk factors include nulliparity, infertility, and a history of breast cancer.[3-8] Factors associated with a reduced risk include high parity, a history of tubal ligation or hysterectomy, and use of oral contraceptives.[8-10] Additional factors for which there is suggestive evidence include the use of talc, fertility drugs, and dietary factors.[11-13]

Mutations in both of the breast cancer genes, *BRCA1* and *BRCA2,* predispose women toward development of ovarian cancer.[14, 15] Ovarian cancer also appears as part of the spectrum of tumors seen in the hereditary nonpolyposis colorectal cancer syndromes.[16] From epidemiologic studies and mutation surveys, it appears that between 5% and 10% of ovarian cancers occur as a result of hereditary predisposition.[14, 15, 17-19] Familial ovarian cancer usually occurs in association with breast cancer (because of mutations in *BRCA1* and *BRCA2*), occasionally as ovarian cancer alone (*BRCA1*[20] or *BRCA2*), or with endometrial, colon, and other solid tumors (hereditary nonpolyposis colorectal cancer syndrome).[21] A family history of ovarian cancer is a strong risk factor for ovarian cancer, increasing with the number of first-degree relatives affected.[2] For families with 1 affected first-degree relative, the estimated lifetime risk increases 1.5 times to 3.6 times.[2-5, 18, 22-25] The population lifetime risk for ovarian cancer in North America is approximately 1:70. The risks rise with additional affected family members.

Families with ≥3 cases of ovarian cancer are generally

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Table I. Histologic characteristics of familial and sporadic ovarian tumors

	Familial (n = 58)	*Nonfamilial (n = 111)*	*Total (N = 169)**
Invasive	54 (93%)	93 (84%)	147
Serous	34	58	92
Endometrioid	7	16	23
Mucinous	5	10	15
Brenner	1	0	1
Clear cell	4	5	9
Mixed	3	4	7
Borderline (total)	4 (7%)	18 (16%)	22
Serous	3	11	14
Mucinous	1	7	8

*One woman did not have information on second-degree relatives, and it is therefore uncertain whether hers was a familial or sporadic case of ovarian cancer. The histologic examination of her tumor revealed a serous adenocarcinoma of the ovary.

considered to represent examples of hereditary ovarian cancer. Although many risk factors for ovarian cancer have been well documented, it is not yet known whether these risk factors apply equally well to the hereditary subgroup. Such knowledge would be important for women with a family history who were seeking information on how to reduce their risk. To address this issue we obtained detailed family histories and other risk factor information for 170 French Canadian women with ovarian cancer and 170 population-based French Canadian control subjects.

Material and methods

Case patients. After institutional review board approval was obtained, a total of 231 French Canadian women with a histologic diagnosis of ovary cancer were found through the gynecologic oncology clinics of 2 large Montreal teaching hospitals in 1995 and 1996. Of these, 48 (21%) were excluded from the study because they died before the interview (21 case patients), they refused to participate or were unavailable for follow-up (12 case patients), or they could not be contacted (15 case patients). A total of 183 case patients were interviewed, representing a response rate of 87% of the eligible living case patients. We later excluded 13 case patients because their ovarian tumors were not of epithelial origin, leading to a final total of 170 patients with ovarian carcinoma or with ovarian tumors of low malignant potential (borderline ovarian tumors). Of these case patients, 70% were interviewed directly in clinics and 30% were interviewed by phone. Each case patient had a histologically confirmed primary invasive carcinoma or a borderline tumor. Pathology records were systematically reviewed and tumors were classified as either serous, mucinous, endometrioid, clear cell, Brenner, or mixed tumors.

Control subjects. Population-based control subjects were obtained through a modified random-digit dialing method. To ensure that the age-distributions of case patients and control subjects were equal, a control subject was selected for each case patient from the page in the telephone directory where the case patient was listed. The names and addresses of 10 persons with the same first 3 digits of the telephone number as the patient were selected. These residences were then contacted by telephone. If there was no answer the number was called 7 more times during the day, the evening, and on weekends before being rejected. Respondents were questioned to determine whether the household contained a woman of self-reported French Canadian origin who matched the index case patient for age within 1 year and who agreed to be interviewed by telephone. If not, the procedure was repeated. A total of 750 households were contacted to obtain the control subjects. Five hundred (66.7%) did not reply or had no eligible women resident, and 10.7% refused to participate. A total of 170 subjects were interviewed.

Questionnaires. Questionnaires were administered in a standardized manner to all case patients and control subjects. The questionnaire was developed, evaluated, and tested within the Quebec Cancer Genetics Network in 1995 and was used at all study centers (Notre-Dame Hospital and Hôtel-Dieu Hospital, Montreal). The 57 questions concerned primarily reproductive factors (age at menarche, age at first childbirth, parity, age and cause of menopause) and medical history (use of hormone replacement therapy and oral contraceptives, tubal ligation, hysterectomy, other surgery), screening histories, and sociodemographic information (smoking, alcohol, education). A detailed family history of cancer was also taken in each case; this inquired about age of diagnosis and type of cancer in all female and male first-, second-, and third-degree relatives.

Statistical methods. In the case-control analysis of the data, relative risk estimates and corresponding 95% confidence intervals were calculated by unconditional logistic regression and maximum likelihood estimation. Multivariate unconditional logistic regression was used to allow for the simultaneous examination of multiple risk factors. Tests of statistical significance were based on differences in the log likelihoods, and all P values are 2-sided. Comparisons between continuously distributed variables were made with the Wilcoxon test. The Fisher exact test was used where appropriate. In the cohort analysis we used a univariate Cox proportional hazards model. The statistical analysis was conducted with the SAS software package (SAS Institute, Inc, Cary, NC).

Results

A total of 170 case patients with ovarian cancer and 170 population-based control subjects were interviewed in 1995. Case patients were born between 1910 and 1969, and their mean age at diagnosis was 53.7 years. Their

Table II. Cumulative incidences of cancer to the age of 70 years among first-degree relatives of case patients and control subjects

			Cox proportional hazards		
Site	*Case relatives (%)*	*Control relatives (%)*	*RR*	*95% CI*	*Significance*
Any					
Female	25.5	15.0	1.84	1.34-2.54	$P = .0002$
Male	19.4	11.8	1.65	1.10-2.49	$P = .015$
Both sexes	22.6	13.5	1.77	1.38-2.28	$P < .0001$
Breast	12.6	3.4	3.68	2.03-6.66	$P < .0001$
Ovary	2.7	2.3	1.32	0.52-3.34	$P = .56$
Prostate	1.4	0.9	1.67	0.28-10.0	$P = .57$
Colon					
Female	3.0	2.4	1.09	0.43-2.74	$P = .86$
Male	5.0	2.1	2.35	0.89-6.18	$P = .08$
Both sexes	4.0	2.3	1.59	0.82-3.06	$P = .17$
Leukemia					
Female	1.5	0.2	6.22	0.75-51.67	$P = .05$
Male	0.40	0.0	∞	—	$P = .15$
Both sexes	1.00	0.1	8.25	1.03-66.01	$P = .02$

RR, Relative risk; *CI,* confidence interval.

mean age at interview was 55.9 years, compared with a mean age of control subjects of 56.7 years. The distribution of the tumor types is presented in Table I.

Family history. We recorded current age, age at death, age at diagnosis of cancer, and cancer site in the relatives of case patients and control subjects. This enabled us to construct 2 historical cohorts, 1 composed of the relatives of case patients and the other of the relatives of control subjects. The cumulative incidence of cancer to the age of 70 years among the relatives of case patients compared with those of control subjects is shown in Table II. The risk of cancer was significantly higher among both female and male relatives of the case patients than those of the control subjects.

The relative risk of any cancer was 1.77 among the relatives of case patients with respect to the relatives of control subjects ($P < .0001$). It is surprising that the relative risks of any cancer were similar in the female and male relatives of the index case patients and control subjects (1.84 vs 1.65). There was a significant excess of breast cancer (relative risk 3.68, $P < .0001$) but not ovarian cancer (relative risk 1.32, $P = .56$) among the relatives of women with ovarian cancer. An eightfold excess of leukemia among the relatives of case patients was observed ($P = .017$).

Families with a total of ≥4 cases of ovarian cancer or of breast cancer in members <55 years old are generally considered to represent examples of hereditary cancer. Seven of the 170 case patients (4.1%) fit into this category. In the following analyses we refer to case patients with a positive family history as having familial cancer (≥1 person with breast cancer diagnosed at <55 years old or 1 other case of ovarian cancer in addition to the proband, on the same side of the family). Fifty-eight women satisfied this criterion. Some of these were due to predisposing genes and others were due to chance aggregations. The disease of case patients with a negative family history is referred to as sporadic.

The average age at onset of the familial case patients was younger than that of the sporadic case patients (51.2 vs 55.2 years; $P = .04$). The average age at onset of the invasive serous tumors was also younger for the familial cancers (54.0 years) than for the sporadic cancers (58.6 years). The distributions of the histologic subtypes were not different for the familial and sporadic invasive tumors (Table I). Only 7% of tumors in the familial group were borderline tumors, whereas 16% in the nonfamilial group were borderline tumors ($P = .09$). Surprisingly, there was no deficit of mucinous tumors among the familial ovarian cancer case patients (5/54 vs 10/93; Table I).

Reproductive, hormonal and other risk factors: Univariate analyses. Although case patients and control subjects did not significantly differ by age at menarche, familial case patients had menarche later (13.3 years) than did sporadic case patients (12.8 years, $P = .038$; Table III). The mean ages at first childbirth were similar for case patients and control subjects, but the mean age at last childbirth was significantly younger for case patients (29.0 years) than for control subjects (30.9 years, $P = .003$), suggesting that late childbirth is protective. Additionally, the mean age at last childbirth was younger for familial case patients (28.2 years) than for sporadic case patients (29.5 years, $P = .19$), suggesting that this protection may be stronger for familial case patients. The interval between first and last childbirth was significantly longer for the control subjects (mean 4.3 years) than for the case patients (mean 3.1 years, $P = .032$). Neither nulliparity nor low parity was a significant risk factor in this study.

As expected, the proportion of ovarian cancer case pa-

Table III. Reproductive factors and oral contraeptive use in control subjects, sporadic and familial ovarian cancer

	Case versus control					*Sporadic versus familial**				
	Control		*Case*			*Sporadic*		*Familial*		
	No.	*Mean*	*No.*	*Mean*	*P*	*No.*	*Mean*	*No.*	*Mean*	*P†*
Age at menarche (y)	170	12.7	169	13.0	.16	111	12.8	57	13.3	.038
Age at menopause‡ (y)	49	48.5	54	48.5	.99	39	48.5	15	48.3	.82
Parity	170	2.1	170	1.8	.16	111	1.8	58	1.8	.95
Age at first childbirth (y)	126	25.2	122	24.6	.28	79	24.8	42	24.3	.51
Age at last childbirth (y)	126	30.9	122	29.0	.0028	79	29.5	42	28.2	.19
Total No. of childbearing years	170	4.3	170	3.1	.032	111	3.3	58	2.8	.47
Use of oral contraceptives (% yes)	170	61.8	170	50.0	.038	111	46.8	58	55.2	.33
Oral contraceptive use duration (y)	152	4.0	154	2.5	.0065	102	2.7	51	2.2	.51
Age at first oral contraceptive use (y)	101	26.3	81	23.1	.0055	49	23.1	31	23.2	.96
Age at last oral contraceptive use (y)	87	32.9	69	28.6	.0016	43	29.5	25	27.6	.27
Tubal ligation (% yes)	170	21.2	169	13.6	.085	110	12.7	58	13.8	.82

Sporadic disease was defined as occurring in patients with no first-, second-, or third-degree relatives with breast cancer diagnosed at <55 years or with ovarian cancer at any age. *Familial* disease was defined as occurring in patients with ≥1 first-, second-, or third-degree relative with breast cancer diagnosed at <55 years or with ovarian cancer at any age.

*In 1 case the familial or sporadic status was unknown.

†The *P* value here compares the means of the sporadic with the familial case patients.

‡Age at menopause was limited to women <55 years old at diagnosis or survey and also to those women who had a natural menopause.

Table IV. Other factors

	Case versus control					*Sporadic versus familial*				
	Control		*Case*			*Sporadic*		*Familial*		
	No.	*% Yes*	*No.*	*% Yes*	*P*	*No.*	*% Yes*	*No.*	*% Yes*	*P**
Alcohol use	170	28.8	170	38.8	.066	111	39.6	58	36.2	.74
Perineal talc use	170	4.7	170	10.6	.064	111	9.91	58	12.1	.79
Breast surgery†	164	7.9	157	15.3	.053	107	19.6	49	6.12	.032
Abdominal surgery	170	38.8	169	36.6	.91	110	43.6	58	32.8	.19

*The *P* value here compares the means of the sporadic with the familial case patients.

†Included as having breast surgery were those who responded with: "nodule," "cyst," or "adenoma." Excluded were those who responded "breast cancers," "breasts implants," and "mammary reduction."

tients who had ever taken oral contraceptives was lower than for control subjects (50% vs 61.8%, $P = .038$). Familial case patients were as likely as sporadic case patients to have ever used oral contraceptives. The mean age at last use of oral contraceptives for all case patients was 28.6 years, compared with 32.9 years for control subjects ($P = .0016$), suggesting that late use of oral contraceptives is protective. Among oral contraceptive users, familial case patients had younger mean age (27.6 years) at last oral contraceptive use than did sporadic case patients (29.5 years), and they used oral contraceptives for a shorter total period (2.2 years) than did sporadic case patients (2.7 years). Although neither of these 2 comparisons were significantly different, they suggest that the protective effect of oral contraceptives may be stronger for familial case patients. In summary, compared with control subjects, case patients began taking oral contraceptives at a younger age but continued for a shorter period. This difference was more marked for the familial case patients. It should be noted that the differences between the subgroups in duration of use of oral contraceptives that we observed were rather small and nonsignificant and should therefore be interpreted cautiously. Significantly more case patients than control subjects had ever had previous breast surgery for nonmalignant conditions (Table IV), and perineal talc application was also more common in case patients. There were no significant differences between case patients and control subjects with respect to anthropometric variables.

Borderline tumors. Women with borderline tumors

Table V. Multivariate model

	All case patients (n = 153) versus control subjects (n = 152)			*Sporadic case patients (n = 101) versus control subjects (n = 152)*			*Familial case patients (n = 51) versus control subjects (n = 152)*		
	RR	*95% CI*	*P*	*RR*	*95% CI*	*P*	*RR*	*95% CI*	*P*
Age at diagnosis*	0.98	0.95-1.01	.14	0.99	0.96-1.02	.33	0.97	0.93-1.00	.06
Age at last childbirth†									
Never pregnant	0.63	0.33-1.19	.16	0.77	0.38-1.59	.48	0.46	0.18-1.21	.12
30-44 y	0.63	0.34-1.15	.13	0.80	0.41-1.58	.52	0.38	0.16-0.90	.027
Age at menarche‡	1.07	0.92-1.25	.40	1.01	0.85-1.20	.92	1.26	1.01-1.57	.041
Age at last oral contraceptive use§									
17-25 y	0.96	0.37-2.48	.93	0.84	0.28-2.55	.76	0.99	0.28-3.51	.99
25-35 y	0.26	0.12-0.57	.0007	0.25	0.10-0.62	.0027	0.26	0.08-0.79	.017
35-43 y	0.24	0.10-0.55	.0008	0.25	0.10-0.64	.0036	0.17	0.036-0.83	.028
Tubal ligation or hysterectomy‖	0.51	0.30-0.88	.016	0.51	0.27-0.95	.033	0.57	0.26-1.27	.17
Talc use¶	2.49	0.94-6.58	.066	2.45	0.85-7.07	.098	3.25	0.85-12.4	.084
Alcohol use									
0-4 drinks/wk#	1.58	0.75-3.33	.23	1.65	0.77-3.79	.23	1.28	0.46-3.61	.64
4-10 drinks/wk#	2.00	0.96-4.17	.063	2.58	1.17-5.68	.019	0.94	0.28-3.10	.92
≥10 drinks/wk#	0.46	0.13-1.69	.24	0.56	0.13-2.37	.43	0.30	0.033-2.71	.28

Ten case patients with no family history of breast or ovarian cancer (sporadic), 7 case patients with family history (familial), and 18 control subjects had missing observations for some of the covariates.

*Age at diagnosis for case patients or current age for control subjects, defined continuously.

†Age at last childbirth is defined categorically. Reference group is last childbirth at 17 to 29 years.

‡Age at first menstrual period, defined continuously.

§Age at last use of oral contraceptives, with baseline no use of oral contraceptives.

‖Tubal ligation or hysterectomy, yes versus no tubal ligation or hysterectomy (without oophorectomy), with baseline of no.

¶Use of talc on perineum, ever versus never, with baseline of never.

#Number of drinks per week, with baseline no drinks per week.

were younger (mean age at diagnosis of 44.4 years) than those with invasive tumors (mean age at diagnosis of 55.1 years). The risk for borderline tumors was less clearly reduced among women who had used oral contraceptives. The mean duration of oral contraceptive intake among women with borderline tumors (4.05 years) was greater than among those with invasive carcinoma (2.31 years). As with invasive cancers, nulliparity was not a risk factor for borderline tumors (relative risk 0.75, $P = .61$).

Multivariate analysis. We incorporated all the significant variables from the univariate analysis into the final unconditional logistic regression model. In the model we also included other variables that had previously been reported to be of possible etiologic relevance but were of borderline significance in the univariate analysis in our study (Table V). The most striking finding in the adjusted analysis is that for all categories of case patients versus control subjects a late age at last use of oral contraceptives is a strong protective factor (relative risk 0.24, $P = .0008$). Various models were assessed, and age at last oral contraceptive use resulted in lower P values and –2 log likelihood values than did age at first use or duration of oral contraceptive use (Table III and data not shown). We therefore used the former parameter in the multivariate analysis. Tubal ligation or hysterectomy (without oophorectomy) was a protective factor (relative risk 0.51, $P = .016$). Use at any time of talc in the perineal region was a positive risk factor (relative risk 2.49), but this result did not reach significance. Moderate alcohol consumption was a positive risk factor for ovarian cancer (relative risk 2.00), but this result was not statistically significant ($P = .063$).

For case patients with a stronger family history of breast or ovarian cancer (≥1 other family members with breast cancer at age <55 years or ovarian cancer at any age), the effects of age at menarche, age at last oral contraceptive use, tubal ligation, and perineal talc application appear to be as strong as, or stronger than, those in the sporadic case patients (Table V). Of particular note is that a late age at last childbirth is highly protective against ovarian cancer in women who have ≥1 other relative with ovarian cancer or breast cancer diagnosed at age <55 years (relative risk 0.38, $P = .027$).

Comment

Family history. We estimate that the hereditary proportion of ovarian cancer in the French Canadian population of Quebec is between 4% and 10%. The lower estimate is based on the observation of 7 families with ≥4 relatives with breast cancer diagnosed at <55 years (or ovarian cancer at any age) among 170 unselected case patients. The higher estimate includes the 17 families with ≥3 such patients (including the index case patient herself). Narod et al[19] used slightly more stringent criteria to estimate that the hereditary fraction of ovarian cancer in southern Ontario was approximately 3% to 7%. They noted that the estimated hereditary proportions of ovarian cancer may be different in other populations,

Table VI. Effect of duration of use of oral contraceptives on risk of ovarian carcinoma

	Duration of oral contraceptive use				
	0-1 y	*1-5 y*	*6-10 y*	*11-25 y*	*Total*
Control subjects	66	35	35	16	152
Case patients	88	36	23	7	154
TOTAL	154	71	58	23	306
Relative risk	1.0	0.77	0.49	0.33	—
95% confidence interval	—	0.44-1.36	0.27-0.91	0.13-0.82	—
P	—	.39	.03	.024	—

both because the frequencies of susceptibility mutations may differ and because varying fertility patterns may lead to differences in average family size. Thus the slightly higher figure reported here, for a province with a relatively small founding population, an established increased incidence of some genetic diseases,[26] and typically large family sizes, is consistent with figures from the Ontario study.

Analyzing the data as a case-control study (rather than as a cohort study), we observed a relative risk of 1.93 (95% confidence interval 0.85 to 4.38) for a family history of ovarian cancer in any first-, second-, or third-degree relative. This figure is comparable to that reported in previous case-control studies. It is apparent that a family history of ovarian cancer is probably the strongest risk factor for ovarian cancer. One strength of our study is that we have included information on breast cancer in the relatives. This is important because breast cancer is more common than ovarian cancer and because breast cancer is more likely to occur in a *BRCA1*- or *BRCA2*-positive pedigree than is ovarian cancer. Thus if we are attempting to determine the hereditary contribution to ovarian cancer, we must question ovarian cancer case patients about both ovarian and breast cancer in their families.

Two genes (*BRCA1* and *BRCA2*) that predispose toward development of ovarian cancer have been identified, and it is possible to directly determine the proportion of ovarian cancer attributable to mutations in these genes. A number of studies have estimated that this proportion is 3% to 26%, depending on the population and the gene studied.[14, 15, 27-31] The absence of a family history does not rule out the possibility of a mutation in *BRCA1* and *BRCA2,* even in cases in which the mutation is known to be of ancient origin. In this study we did not analyze the case patients for mutations, but it will be interesting to determine the frequencies of *BRCA1* and *BRCA2* mutations among French Canadian women with ovarian cancer.

We used a historical cohort approach to analyze the data; this increases the information that can be gathered from the pedigree. In this study there was an overall excess of cancer at all sites in men and women, with leukemia (relative risk 8.25, 6 cases observed, 1 case expected) showing the largest relative risk (Cox proportional hazard relative risk, Table II). Among female first-degree relatives of case patients, breast cancer was seen in significant excess compared with relatives of control subjects (relative risk 3.68, $P = .0001$). Interestingly, there was no significant excess of ovarian cancer (relative risk 1.32, $P = .56$). Among male relatives the relative risk for colon cancer was 2.35 (13 cases observed, 6 expected, $P = .075$). There was no excess of colon cancer among female relatives. Previous case-control studies of ovarian cancer have shown that ovarian,[2-5, 18, 22-25] breast,[5, 25] colon,[4] pancreatic,[2] and prostate cancers[4] are all significantly overrepresented among the relatives of women with ovarian cancer. However, only the Utah study[2] was able to study distant relatives systematically, and only our study used a historic cohort approach to calculate the relative risks in a proportional hazards model. In a study of the first-degree relatives of women with breast, ovarian, or endometrial cancer, a significant excess of ovarian and breast cancer was seen among the relatives of women with either breast or ovarian cancer.[32] In fact, the risk of breast cancer was not significantly different for women with a family history of ovarian cancer rather than breast cancer. A record linkage study from Iceland[33] also showed a significant 90% excess of ovarian cancer among the first-degree relatives of women with breast cancer. However, this was not seen in the United Kingdom Office of Population, Censuses and Surveys studies of ovarian and breast cancer.[34, 35] Thus, considering all studies together, it is likely that a personal history of ovarian cancer is significantly associated with a family history of breast or ovarian cancer, and a family history of breast cancer should always be sought in any woman at risk for ovarian cancer.

Oral contraception, reproductive risk factors, and tubal ligation. The most striking findings in our study are the protective effects of late use of oral contraceptives, a long interval between first and last live childbirth, and a late age at last childbirth (Tables III and V). Many other studies have shown oral contraceptive use to be inversely associated with ovarian cancer. Hankinson et al[10] reana-

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lyzed 20 epidemiologic studies and found a summary relative risk of 0.64 (95% confidence interval 0.57 to 0.73) for use of oral contraceptives at any time. They also found that the effect was strongest in women who had >5 years of use. By contrast, in a reanalysis of 12 US case-control studies no extra protective effect after 6 years of use was found.[8] In our study we found that an increasing duration of use of oral contraceptives was associated with a decreasing risk (relative risk 0.89, $P = .00013$) for each year of use and, unlike Whittemore et al,[8] we observed no diminution of effect with increasing number of years of use. In fact we noted a marked trend toward increasing protection against ovarian carcinoma with increasing duration of use of oral contraceptives (Table VI). Among women who had used oral contraceptives for >6 years, there was a suggestion that oral contraceptive use was more protective for those who continued use beyond 10 years rather than stopping after 10 years (the relative risk for 11 to 25 years of use vs 6 to 10 years of use was 0.67, $P = .61$). Compared with those who took oral contraceptives for <5 years, those with 11 to 25 years of use resulted in a relative risk of 0.43 ($P = .10$). Hormone replacement therapy has not been shown to be a risk factor for ovarian cancer,[8] and this finding was confirmed in our study.

An early age at menarche is a risk factor for ovarian cancer, but the risk increase is relatively small, ranging from 1.2 to 1.5 when comparing commencement of menarche at <12 years with commencement at >15 years.[36] In our study case patients and control subjects did not significantly differ with respect to age of menarche, but familial case patients had menarche later (13.3 years) than did sporadic case patients (12.8 years, $P = .038$; Table III). This difference was maintained in the multivariate analysis (Table V). High parity is protective,[8] but in this study we showed that in an adjusted analysis a late age at last childbirth and a long interval between first and last live childbirth were more important than the total number of pregnancies or the age at first childbirth (Table V). A large, nested case-control study from Sweden showed that a late age at first childbirth was more protective than high parity.[7] In the review by Parazzini et al[36] of 16 previous studies, however, only 1 suggested that a late first childbirth was more protective than an early first childbirth. It is intriguing that in a study of *BRCA1* carriers a late age at last childbirth was protective against ovarian cancer and there was no increased risk associated with nulliparity.[37] There also was no protective effect of high parity against familial ovarian cancer in the Utah study.[2]

We found that tubal ligation or hysterectomy (without oophorectomy) was protective (adjusted relative risk 0.51, $P = .016$; Table V). There was no change in the point estimates of the risk reduction among those with a family history of breast or ovarian cancer compared with those without a family history. Tubal ligation has previously been associated with a reduced risk of ovarian cancer. In a large, prospective study, Hankinson et al[9] observed a strong inverse association between tubal ligation and ovarian cancer that persisted after adjustment for age, oral contraceptive use, parity, and other ovarian cancer risk factors (relative risk 0.33, 95% confidence interval 0.16 to 0.64). They also noted a weaker inverse association between simple hysterectomy and ovarian cancer. The protective effect of hysterectomy is increased if the operation is carried out at a younger age.[8, 9] Whether tubal ligation has its effect by preventing external carcinogenic agents from reaching the ovary or by alteration of the local environment of the ovary is not known.

Alcohol and talc as potential risk factors for ovarian cancer. In this study we found that moderate alcohol consumption (between 4 and 10 drinks per week compared with no alcohol use) was nonsignificantly associated with ovarian cancer (relative risk 2.00, $P = .063$). Other studies have also reported that moderate to high alcohol consumption is nonsignificantly associated with ovarian cancer.[12, 13, 38] Perineal talc use was a nonsignificant risk factor in our study (relative risk 2.49, $P = .064$). Talc has been previously implicated in the development of ovarian cancer.[11, 13] Although there are reports of talc embedded in human ovarian tissue and of talc migrating through the human female reproductive tract, the literature reviewed does not provide any convincing evidence that pure cosmetic talc, when used as intended, presents a health risk to women.[11, 39, 40]

Familial and sporadic ovarian cancer: Are the risk factors the same? Early detection of ovarian cancer is difficult. No screening procedure has been shown to reduce mortality rates, and no single test has yet proved to be practical for population screening. However, it is possible that those at higher risk may be more likely to benefit from screening procedures, because the positive predictive value of a screening test depends in part on the prevalence of the disease in the population under study. The inherited fraction of ovarian cancer in this study is between 4% and 10%, and perhaps we should direct our preventive and early detection efforts toward these women at higher risk. Women with a family history of ovarian cancer are more likely to carry mutations in highly penetrant cancer-predisposing genes. The findings of this study raise the possibility that preventive measures such as oral contraceptive use and tubal ligation may be effective in preventing ovarian cancer in persons at high risk. The findings that a late age of last use of oral contraceptive, a late age of last childbirth and a prolonged interval between first and last childbirth are protective suggest that the timing of the intervention is important. For example, hysterectomy appears to be most protective when it is carried out in the early to middle

40s.[8, 9] Anovulation appears to be a protective state for ovarian cancer, but the protective effects of oral contraceptive and pregnancy may change with time.[41]

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Exhibit 37

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GENITAL TALC EXPOSURE AND RISK OF OVARIAN CANCER

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Epidemiologic studies have suggested an increased risk for ovarian cancer associated with the use of talcum powder in genital hygiene, but the biologic credibility of the association has been questioned. We conducted a population-based case-control study in eastern Massachusetts and New Hampshire involving 563 women with newly diagnosed epithelial ovarian cancer and 523 control women selected either by random digit dialing or through lists of residents. Use of body powders was assessed through personal interview and the exposure odds ratio (OR) for the use of talc in genital hygiene was calculated. Cases were more likely than controls (45% *vs.* 36%) to have used talc as a body powder in some manner, and the excess was confined to patients who used talc on the perineum directly or as a dusting powder to underwear or sanitary napkins. Relative to women who never used body powder or used it only in non-genital areas, the OR (and 95% confidence interval) associated with genital exposure to talc was 1.60 (1.18 and 2.15) after adjustment for age, study location, parity, oral contraceptive use, body mass index and family history of breast or ovarian cancer. Exposure prior to rather than after a first livebirth appeared to be more harmful, and the association was most apparent for women with invasive serous cancers and least apparent for those with mucinous tumors. We conclude that there is a significant association between the use of talc in genital hygiene and risk of epithelial ovarian cancer that, when viewed in perspective of published data on this association, warrants more formal public health warnings. *Int. J. Cancer* 81:351–356, 1999.
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An association between the use of talc in genital hygiene and ovarian cancer was first examined in an epidemiologic study in 1982 (Cramer *et al.,* 1982). An elevated odds ratio for genital talc exposure was observed in this study, in 8 of the largest subsequent epidemiological studies (Whittemore *et al.,* 1988; Booth *et al.,* 1989; Harlow *et al.,* 1992; Chen *et al.,* 1992; Purdie *et al.,* 1995; Shushan *et al.,* 1996; Cook *et al.,* 1997; Chang and Risch, 1997) and in a study of borderline tumors (Harlow and Weiss, 1989). Only 3 smaller studies reported a null association (Hartge *et al.,* 1983; Rosenblatt *et al.,* 1992, Tzonou *et al.,* 1993). Despite this consistency, the association is still viewed with skepticism based upon weak odds ratios, poor dose-response relationships and an incomplete understanding of the biological mechanism by which talc might lead to ovarian cancer. We have completed a large population-based case-control study of ovarian cancer which offers new perspectives on the validity of the talc and ovarian cancer association.

MATERIAL AND METHODS

We conducted a population-based case-control study of women with newly diagnosed ovarian cancer who resided in eastern Massachusetts (MA) or New Hampshire (NH). Women with ovarian cancer were identified through hospital tumor boards and statewide cancer registries. Between 5/92 and 3/97, 1,080 cases of ovarian cancer were identified. After excluding 203 cases who had died or moved, had no telephone, did not speak English or had a non-ovarian primary tumor after review, 877 women remained eligible. Physicians denied permission to contact 126 (14%) of these women, and 136 cases (16%) declined to participate. Our analysis is based upon data from 563 cases with epithelial ovarian cancer, including those with tumors of borderline malignancy.

We identified control women using random digit dialing (RDD) in which the sampling unit for an interviewed case comprised the 99 telephone numbers generated from the first 5 digits of her telephone number plus all remaining combinations of the last 2 digits (excluding the case's own number). These numbers were listed in random order and called to screen households for potential controls who were within 4 years of the age of the case. Excluding business and non-working numbers, approximately 5,400 calls yielded 10% of households in which the household member declined to provide a household census and 80% of households in which an age and sex matched control for a case could not be made or a potential control was ineligible because of a prior oophorectomy. Of the remaining 10% of households screened with a potential eligible control, 72% agreed to participate. RDD proved inefficient for identifying controls over age 60 in MA since a substantially greater number of households needed to be screened to obtain an older control. Except in NH where complete listings of residents were unavailable, we chose to identify older controls in MA by randomly selecting women through use of lists (townbooks) of all residents in towns by name, age, and address according to precinct. We matched older controls to cases by community and age within 4 years based on the townbooks. Of 328 sampled townbook controls, 21% could not be reached, 18% were ineligible and 30% declined to participate. This analysis includes a total of 523 RDD and townbook controls.

In introducing the study to potential cases and controls, specific hypotheses including the talc association were not discussed. After written informed consent, we assessed demographic information, menstrual and reproductive history, medical and family history and personal habits using an in-person interview. We assessed exposures occurring prior to a ''reference date,'' defined as 1 year before the date of diagnosis for cases and the date of interview for controls. We asked whether women had ''regularly used talc, baby, or deodorizing powders dusted or sprayed'' to feet, arms or other non-genital areas, to the genital or rectal area, on sanitary napkins, or on underwear, with the latter 3 methods defined as ''genital exposure'' and either no use or use in non-genital areas defined as ''no genital exposure.'' A husband's use of powder in his genital area was also assessed. Age at first use, types of powder(s) used, applications per month and total years of use in genital hygiene were assessed in talc users. We did not assess potential talc exposure from diaphragms or condoms, exposures not found to be associated with ovarian cancer in our previous studies (Cramer *et al.,* 1982; Harlow *et al.,* 1992).

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TABLE I – PERINEAL TALC EXPOSURE[1] IN RELATION TO OVARIAN CANCER RISK BY CHARACTERISTICS OF STUDY PARTICIPANTS

	Cases		Controls		Age-adjusted[2] OR	(95% C.I.)
	Total	Talc exposure (%)	Total	Talc exposure (%)		
Age						
<50	266	66 (24.8)	262	43 (16.4)	1.68	(1.09, 2.58)
≥50	297	86 (29.0)	261	52 (19.9)	1.64	(1.11, 2.43)
Study center						
MA	433	126 (29.1)	411	85 (20.7)	1.56	(1.14, 2.14)
NH	130	26 (20.0)	112	10 (8.9)	2.49	(1.14, 5.45)
Education						
12	218	58 (26.6)	171	28 (16.4)	1.79	(1.08, 2.97)
>12	344	93 (27.0)	352	67 (19.0)	1.59	(1.10, 2.27)
Marital status						
Never married	110	31 (28.2)	61	10 (16.4)	1.77	(0.78, 4.00)
Ever married	453	121 (26.7)	462	85 (18.4)	1.62	(1.18, 2.22)
Religion						
Jewish	54	18 (33.3)	44	10 (22.7)	1.69	(0.68, 4.18)
Non-Jewish	509	134 (26.3)	479	85 (17.8)	1.63	(1.20, 2.22)
Weight						
<140	237	57 (24.0)	247	40 (16.2)	1.60	(1.02, 2.53)
≥140	326	95 (29.1)	275	55 (20.0)	1.65	(1.13, 2.42)
Use of OCs (months)						
<3 or never	334	98 (29.3)	247	52 (21.0)	1.55	(1.06, 2.28)
≥3	229	54 (23.6)	276	43 (15.6)	1.67	(1.07, 2.61)
Number of liveborn children						
0	185	55 (29.7)	106	20 (18.9)	1.65	(0.92, 2.98)
1–2	212	49 (23.1)	209	34 (16.3)	1.56	(0.95, 2.54)
3+	166	48 (28.9)	208	41 (19.7)	1.69	(1.04, 2.75)
Prior tubal ligation						
No	488	135 (27.7)	437	76 (17.4)	1.80	(1.31, 2.47)
Yes	75	17 (22.7)	86	19 (22.1)	0.98	(0.46, 2.08)
Prior hysterectomy						
No	529	139 (26.3)	487	88 (18.1)	1.60	(1.18, 2.16)
Yes[3]	34	13 (38.2)	36	7 (19.4)	2.61	(0.88, 7.78)
Family history of breast or ovarian cancer						
No	481	132 (27.4)	462	87 (18.8)	1.59	(1.17, 2.17)
Yes	82	20 (24.4)	61	8 (13.1)	2.21	(0.89, 5.48)

OR: odds ratio; CI: confidence interval; OCs: oral contraceptives.–[1]Sources of perineal talc exposure include dusting of underwear, diaphragms, sanitary napkins and/or dusting of genital area.–[2]Adjusted for age as a continuous variable.–[3]Excludes those with tubal ligation prior to hysterectomy.

For all cases studied, we reviewed pathology reports and sought slides in any instance where there was a discrepancy between histologic description and final diagnosis. After completing the review, cases were grouped according to the following histologic categories: serous cancers (including serous cystadenocarcinomas and surface papillary carcinomas), mucinous cancers, endometrioid and clear cell cancers, including mixed mesodermal or mixed epithelial with an endometrioid or clear cell component) and undifferentiated or other cancers. According to Young *et al.* (1994), serous tumors tend to be either borderline or invasive and seldom display a mixture while borderline and invasive grades often intermingle within other histologic types, especially the mucinous tumors. Based on this tendency, only serous borderline tumors were distinguished from invasive cancers when considering odds ratios by histologic type and grade.

Since matching was performed as the most convenient means for selecting controls comparable to cases in age and geographic locale and not as the principal means of controlling for confounding, matching was not preserved in the analysis. We analyzed our data by constructing frequency counts of cases and controls by study variables and by calculating crude odds ratios (OR). We then used unconditional logistic regression to adjust for the matching variables including age (continuous), study site (MA, NH), body mass index (continuous), which might have influenced likelihood of using body powder, and for variables strongly linked to ovarian cancer risk such as parity (0, 1), oral contraceptive use (never or <3 months, ≥3 months) and family history of breast or ovarian cancer (no, yes) and tubal ligation (no, yes). Most analyses were performed by using the SAS system (SAS Institute, Cary, NC). Tests for linear trend were performed using the likelihood ratio test with continuous forms of the talc variables. Frequency counts from studies included in our review of published studies were entered into STATA (College Station, TX) to compute crude and combined odds ratios.

RESULTS

Table I summarizes data regarding how cases and controls differed demographically and by known risk factors for ovarian cancer, how these same variables influenced genital talc exposure among controls and how the association between talc use in the genital area and ovarian cancer varied among strata. Controls were more likely than cases to have gone beyond high school, to have married, to have had children and to have used oral contraceptives. In examining the frequency of talc use among controls, only study location significantly influenced likelihood of genital talc exposure. Women from New Hampshire were less likely to have used talc in the genital area compared to women from Massachusetts. Ovarian cancer cases in almost all strata were more likely to have used powder genitally compared to controls, with corresponding elevated odds ratios. A notable exception was the lack of an association between talc use and ovarian cancer among women who reported having had a tubal ligation.

Table II shows adjusted odds ratios by manner, type and frequency of powder use. A greater percentage of cases had regularly used powder in some manner compared to the controls.

TABLE II – ADJUSTED ODDS RATIOS FOR OVARIAN CANCER ASSOCIATED WITH TYPES AND FREQUENCY OF POWDER USE

Type of personal use	Cases Number (%)	Controls Number (%)	Adjusted OR[1]	(95% C.I.)
No personal use	312 (55.4)	334 (63.9)	1.0	
Use, non-genital areas	99 (17.6)	94 (18.0)	1.08	(0.77, 1.50)
Use, dusting perineum	71 (12.6)	51 (9.8)	1.45	(0.97, 2.18)
Use, dusting sanitary napkin	20 (3.6)	12 (2.3)	1.45	(0.68, 3.09)
Use, dusting underwear	8 (1.4)	6 (1.2)	1.21	(0.40, 3.64)
Multiple uses genital area	53 (9.4)	26 (5.0)	2.15	(1.30, 3.57)
Genital use				
No personal genital exposure	411 (73.0)	428 (81.8)	1.0	
Any personal genital exposure	152 (27.0)	95 (18.2)	1.60	(1.18, 2.15)
Longest used type of powder[2]				
No genital use	411 (73.4)	428 (81.8)	1.0	
Talc	148 (26.4)	92 (17.6)	1.69	(1.26, 2.27)
Cornstarch	1 (0.2)	3 (0.6)	0.31	(0.03, 3.01)
Husband use[3,1]				
No	291 (87.6)	346 (92.0)	1.0	
Yes	41 (12.4)	30 (8.00)	1.52	(0.92, 2.52)
Frequency of use per month[4]				
<30	64 (11.5)	28 (5.4)	2.21	(1.37, 3.56)
30–39	59 (10.6)	51 (9.8)	1.17	(0.78, 1.76)
40+	23 (9.8)	15 (2.9)	1.57	(0.80, 3.10)

[1]Adjusted for age (continuous), study center (MA, NH), tubal ligation (ever, never), BMI (continuous), parity (0, ≥1), OC use (<3 months, ≥3 months), and primary relative with breast or ovarian cancer (yes, no) and other categories of genital talc use, except where noted.–[2]Adjusted for age (continuous), study center (MA, NH), and tubal ligation (ever, never) and other powder.–[3]Among married women with no personal genital talc use.–[4]Total of all uses in the genital area.

Relative to those with no use of a body powder, those who used powder only in non-genital areas did not have an increased risk of ovarian cancer [OR=1.08 (0.77 and 1.50)]. However, elevated ORs and (95% CI) were observed for women who directly powdered the genital or rectal area [1.45 (0.97 and 2.18)]; who dusted sanitary napkins: 1.45 (0.68 and 3.09); who dusted underwear [1.21 (0.40 and 3.64)] and who used powder in multiple ways in the genital area [2.15 (1.30 and 3.57)]. There was a significant excess of cases who regularly used powder in some manner in the genital area, and the adjusted OR was similar whether the non exposed referent group was considered to be women with no use of talc anywhere [OR= 1.58, (1.16 and 2.16)] or women with no genital use including those who used it as a body powder in non-genital areas [OR= 1.60 (1.18 and 2.15)]. Few of the women in our study reported use of cornstarch rather than a talc-based powder leading to an imprecise and non-significant OR for ovarian cancer risk associated with its use in the genital area. Among married women who never personally used talc in the genital area, there was an increase of borderline significance in ovarian cancer risk for women whose husbands had used talc in their genital area [OR=1.52 (0.92, 2.52)]. When we examined all methods of genital talc use (except exposure from a husband), we found that most of those who used talc had 30 or more applications per month, but there was no apparent trend for increasing risk for ovarian cancer with increasing number of monthly applications.

Table III examines risk for ovarian cancer associated with ordinal categories related to duration or intensity of talc exposure in the genital area relative to women who never used talc or who used it only in non-genital areas. No clear linear trend was apparent in ORs for categories of age at first use, years of use or total applications. To examine dose response, each of these variables was used as a continuous variable in multivariate models. Linear trends were significant only in those models that included women who were not exposed. To duplicate an analysis performed in a previous report (Harlow *et al.,* 1992), we examined total applications censored by excluding use after closure of the female tract or during non-ovulatory years. Although the ORs for the categories displayed a trend, once again only the multivariate model including the non-genitally exposed revealed a significant trend.

Table IV presents a more detailed analysis of the effect of genital use of talc in women who had no pregnancies at all, in women who had a pregnancy not resulting in a liveborn and in women with a liveborn pregnancy. In the latter 2 groups, we examined risk for ovarian cancer with the timing of talc use in relation to the first pregnancy. Genital talc use that began after a first pregnancy appeared to be associated with lower risk compared to use which began before the first pregnancy. The effect was more apparent among those with a liveborn. Eighty-five of 374 parous cases used at least some talc prior to their first liveborn compared to 64 of 416 parous controls, leading to an adjusted OR (95% CI) of 1.58 (1.10 and 2.29). In contrast, 8 of 378 parous cases used talc only after their first livebirth compared to 10 of 417 parous controls, leading to an adjusted OR(95% CI) of 0.97 (0.38 and 2.50) for ovarian cancer associated with talc use after a first livebirth.

Table V shows the average age and use of genital talc for all controls and for cases by histologic type of ovarian cancer. Average age differed by histologic type but did not account for the differences in ORs. The odd ratio for genital talc use was greatest (and significant) for invasive serous tumors and less than 1 only for mucinous tumors (invasive and borderline combined) after adjustment for age and other covariates.

DISCUSSION

Consistent with four recent case-control studies of ovarian cancer (Purdie *et al.,* 1995, Sushan *et al.,* 1996, Cook *et al.,* 1997, Chang and Risch, 1997), our results demonstrate a significant association between the use of talc in genital hygiene and risk for ovarian cancer. In our discussion, we will examine whether this association satisfies traditional criteria for a causal association including consistency and strength of the association, potential biases, dose response and biological credibility.

Figure 1 summarizes data on risk for ovarian cancer with any genital use of talc from 14 case-control studies including this one. The combined odds ratio and 95% CI is 1.36 (1.24 and 1.49), which is statistically significant. Odds ratios deviating most from the pooled value were observed in the smaller studies, and the test for heterogeneity was not significant ($p=0.085$). Thus, the criteria for

TABLE III – ADJUSTED ODDS RATIOS FOR OVARIAN CANCER ASSOCIATED WITH GENITAL USE OF TALC

Type of exposure	Cases Number (%)	Controls Number (%)	Adjusted OR[1]	(95% C.I.)
No genital use	411 (73.0)	428 (81.8)	1.0	
Age at first use				
<20	97 (17.4)	67 (12.8)	1.46	(1.03, 2.07)
20–25	36 (6.5)	18 (3.4)	1.87	(1.03, 3.39)
>25	13 (2.3)	9 (1.7)	1.54	(0.64, 3.72)
p-value for linear trend is 0.504 excluding non-exposed.				
Years of use				
<20	55 (9.9)	31 (5.9)	1.86	(1.16, 3.00)
20–30	32 (5.8)	26 (5.0)	1.33	(0.76, 2.30)
>30	59 (10.6)	37 (7.1)	1.44	(0.91, 2.26)
p-value for linear trend is 0.477 excluding non-genitally exposed and 0.062 including non-genitally exposed.				
Total applications				
<3000	51 (9.2)	27 (5.2)	1.84	(1.12, 3.03)
3000–10,000	36 (6.5)	28 (5.4)	1.43	(0.84, 2.41)
>10,000	59 (10.6)	39 (7.5)	1.43	(0.92, 2.22)
p-value for linear trend is 0.164 excluding non-genitally exposed and 0.472 including non-genitally exposed.				
Applications censored[2]				
<3000	59 (10.6)	41 (7.8)	1.54	(1.01, 2.35)
3000–10,000	51 (9.2)	31 (5.9)	1.72	(1.08, 2.76)
>10,000	36 (6.5)	20 (3.8)	1.80	(1.02, 3.18)
p-value for linear trend is 0.675 excluding non-genitally exposed and 0.022 including non-genitally exposed.				

[1]Adjusted for age (continuous), study center (MA, NH), BMI (continuous), primary relative with breast or ovarian cancer (yes, no), parity (0, ≥1), OC use (<3 months, ≥3 months), tubal ligation, and other categories of genital talc use, except where noted.–[2]Excludes applications following hysterectomy or tubal ligation and applications during pregnancy and periods of OC use. Adjusted for age (continuous), study center (MA, NH), BMI (continuous) and primary relative with breast or ovarian cancer (yes, no).

TABLE IV – EVER USE OF TALC IN THE GENITAL AREA IN RELATION TO PREGNANCY AND CHILDBIRTH

Group	Cases Total	Cases Number exposed	(%) exposed	Controls Total	Controls Number exposed	(%) exposed	Adjusted OR	95% C.I.
Nulligravid[1]	145	42	(29.0)	82	17	(20.7)	1.48	(0.76, 2.86)
Nulliparous[1] prior to first pregnancy	40	13	(32.5)	24	3	(12.5)	2.80	(0.64, 12.20)
Nulliparous[1] only after first pregnancy	40	2	(5.0)	24	1	(4.2)	1.24	(0.10, 15.32)
Parous[1] prior to first livebirth	374	85	(22.7)	416	64	(15.4)	1.58	(1.10, 2.29)
Parous[2] only after first livebirth	378	8	(2.12)	417	10	(2.40)	0.97	(0.38, 2.50)

[1]Adjusted for age (continuous), study center (MA, NH), BMI (continuous) and primary relative with breast or ovarian cancer (yes, no).–[2]Adjusted for age (continuous), study center (MA, NH), BMI (continuous), primary relative with breast or ovarian cancer (yes, no) and tubal ligation.

TABLE V – HISTORY OF GENITAL TALC USE AND ASSOCIATED ODDS RATIOS BY HISTOLOGIC TYPE AND GRADE

Histologic type/grade	Total	Average age	Any use of genital talc	No use of genital talc	Adjusted OR[1]	(95% CI)
Controls	523	49.3	95	428	1.0	
Histologic type/grade						
Serous borderline	86	41.8	23	63	1.38	(0.82, 2.31)
Serous invasive	229	54.5	72	157	1.70	(1.22, 2.39)
Mucinous	83	46.7	16	67	0.79	(0.44, 1.40)
Endometrioid/clear cell	130	53.9	31	99	1.04	(0.67, 1.61)
Undifferentiated	35	52.9	10	25	1.44	(0.67, 3.08)

[1]Adjusted for age (continuous), study center (MA, NH), primary relative with breast or ovarian cancer (yes, no), BMI (continuous), parity (0, ≥1), OC use (<3 months, ≥3 months) and tubal ligation (ever, never).

consistency of the association appear to be satisfied. A summary odds ratio of 1.36 suggests that between 10 and 11% of ovarian cancers in these populations are attributable to the genital use of talc depending upon whether the average control exposure of 36% or average case exposure of 43% is considered.

Despite the consistency noted above, the relatively weak odds ratios observed could reflect potential biases, especially recall and confounding. Recall bias is possible because talc exposure in these studies is based on personal recollection. However, recall bias seems more likely to affect exposures that have occurred over a short term than those that have occurred over a long term. Since average duration of talc use exceeded 20 years in both cases and controls in our current study, genital talc exposure may be less likely to be subject to recall bias. Furthermore, if publicity regarding the association correlated with selective recall, one might expect a trend for cases from more recent studies to report higher

Risk of ovarian cancer with perineal exposure to talc from key epidemiologic studies.

Author	Cases Total n	Cases Exposed (%)	Controls Total n	Controls Exposed (%)	Crude OR (95% CI)
Cramer *et al.* (1982)	215	(42.8)	215	(28.4)	1.9 (1.3-2.8)
Hartge *et al.* (1983)	135	(49.6)	171	(58.5)	0.7 (0.4-1.1)
Whittemore *et al.* (1988)	188	(52.1)	539	(46.0)	1.3 (0.9-1.8)
Harlow and Weiss (1989)	116	(42.2)	158	(40.5)	1.1 (0.7-1.7)
Booth *et al.* (1989)	217	(65.0)	434	(59.0)	1.3 (0.9-1.8)
Harlow *et al.* (1992)	235	(48.5)	239	(39.3)	1.4 (1.0-2.1)
Rosenblatt *et al.* (1992)	77	(87.0)	46	(88.0)	1.0 (0.3-3.0)
Chen *et al.* (1992)	112	(6.2)	224	(2.2)	2.9 (0.9-9.4)
Tzonou *et al.* (1993)	189	(3.2)	200	(3.5)	0.9 (0.3-2.7)
Purdie *et al.* (1995)	824	(56.7)	860	(52.0)	1.2 (1.0-1.5)
Shushan *et al.* (1996)	200	(10.5)	408	(5.6)	2.0 (1.0-3.6)
Cook *et al.* (1997)	313	(50.8)	422	(39.3)	1.6 (1.2-2.1)
Chang and Rish (1997)	450	(44.0)	564	(35.6)	1.4 (1.1-1.8)
Cramer *et al.* (1999)	563	(27.0)	528	(18.2)	1.7 (1.2-2.2)
Summary odds ratio (95% confidence interval)					1.4 (1.2-1.5)

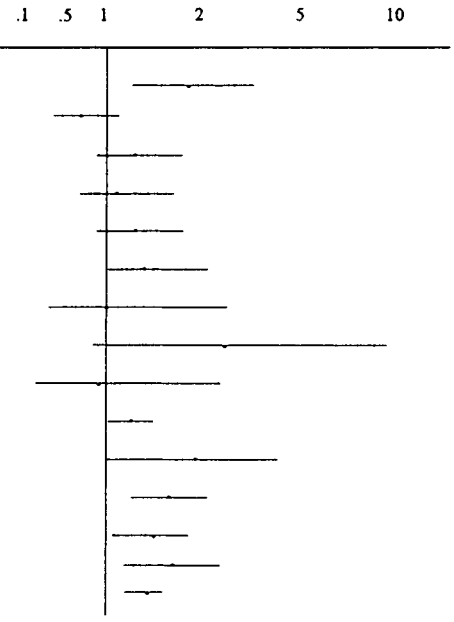

FIGURE 1 – Exposure rates, crude odds ratios and confidence intervals for case-control studies of genital talc use and ovarian cancer.

exposure rates, but the exposure rates noted in Figure 1 do not suggest this is the case. It also seems reasonable that selective recall would lead to cases reporting all types of talc exposure more frequently than controls, but our study found that cases did not report a significant excess of talc use in non-genital areas compared to controls. Finally, if recall accounted for the association, one would expect little variation in the odds ratios by histologic type of ovarian cancer which appears not to be the case from Table V. Our study found the greatest risk to be associated with invasive serous tumors, OR=1.70 (1.22 and 2.39). Cook *et al.* (1997) found talc use to be most strongly associated with serous and unclassified cancers, although Chang and Risch (1997) found endometrioid cancers to be more strongly linked with talc use.

Regarding potential bias from confounding, we found no evidence that genital talc exposure varied by key risk factors for ovarian cancer such as age, parity or OC use and little variability of the association by these and other variables (Table II). Chang and Risch (1997) adjusted for age, parity, breastfeeding, oral contraceptive use, tubal ligation or hysterectomy and family history and also found the association to persist. Characteristics such as body odor or excessive perspiration might represent subtle constitutional features that might predispose to both talc use and ovarian cancer, but adjusting for BMI should control for these effects. In addition, 2 previous studies (Cook *et al.*, 1997; Chang and Risch, 1997), and our current study found no evidence of elevated risk associated with genital use of a cornstarch based-powder, although in all of these studies the exposure was infrequent and the OR and confidence interval was wide. Further studies would be valuable since this observation suggests that type of powder used may be more important than underlying reason for use.

The most obvious weakness in the argument for biologic credibility of the talc and ovarian cancer association is the lack of a clear dose response. Most talc and ovarian cancer studies that have addressed dose response, including this one, have failed to demonstrate consistent dose response relationships with measures of the intensity of the exposure, especially when the trend is examined among users only. In attempting to address this weakness, we point out that it is difficult to quantify the amount of powder actually used and degree of perineal dusting that might constitute an ''application of talc.'' Another factor that may affect the dose-response relationship is whether use occurred at a time when the female tract was open. There is evidence from several studies that the talc/ovarian cancer association is modified by closure of the female tract as a result of tubal ligation or hysterectomy (Harlow *et al.*, 1992; Chang and Risch, 1997; Green *et al.*, 1997). We have also proposed that talc use during periods of ovulation may carry greater risk, based on the hypothesis that ovarian surface epithelial disruption and repair accompanying ovulation might allow talc to become entrapped within the inclusion cysts that form with ovulation.

Our current study also suggests that a term pregnancy may affect the relationship between talc and ovarian cancer in a manner that may be independent of ovulation. We observed that the association between talc and ovarian cancer was more apparent in women who used talc prior to a first liveborn pregnancy compared to those who used it only after a first liveborn pregnancy. This may suggest that ovarian tissue that has not (yet) gone through a pregnancy may be more susceptible to talc-induced damage than tissue that has undergone a pregnancy. A possible biologic explanation for this may involve an ovarian change, known as decidual reaction, that occurs during pregnancy. The decidual reaction refers to differentiation of stromal cells that occurs primarily in the endometrium of the pregnant uterus but which also may be seen in the fallopian tubes, pelvic peritoneum and ovarian surface (Herr *et al.*, 1978). Studies to determine whether the decidual reaction alters the susceptibility of ovaries (or pelvic peritoneum) to talc-induced damage may be informative.

Although we do not know precisely how use of talc in the genital area might induce ovarian cancer, some key elements supporting the biologic plausibility of the association have been established. It has been demonstrated that inert particles contaminating the vagina can reach the ovaries (Venter and Iturralde, 1979). Talc has been found in both normal and malignant ovarian tissue (Henderson *et al.*, 1979), although Heller *et al.* (1996) reported a poor correlation between the amount of talc in the ovaries and personal history of talc use. The patency of the female tract and the nature of ovarian cancer as a surface epithelial (mesothelial) lesion make the ovary a target for foreign body carcinogenesis. Indeed, human ovarian cancer has been demonstrated to be a consequence of occupational asbestos exposure (Keal, 1960). Talc, as a chemical relative of asbestos, appears able to induce histologic changes that are similar to those of asbestos, at least in the lungs (Kleinfeld et al., 1967). Biologic credibility for an association would be strengthened by an animal model, but an experiment capturing all of the potential factors in the human ''model'' would be very difficult. These elements include chronicity of the exposure, anatomic and physiologic uniqueness of women, effects of pregnancy and potential spread through coitus (as suggested by our finding related to ovarian cancer risk associated with a husband's use of talc). Rodent models seem poorly suited to address these issues because of their infrequent ovulation and the fact that the rodent ovary is encased in a bursal sac.

In summary, we have demonstrated a consistent association between talc and ovarian cancer that appears unlikely to be explained by recall or confounding. The dose-response relationship is weak but improved by considering factors such as closure of the female tract, ovulation and exposure prior to pregnancy, and we have outlined a plausible biologic rationale for this association. We estimate that avoidance of talc in genital hygiene might reduce the occurrence of a highly lethal form of cancer by at least 10%. Balanced against what are primarily aesthetic reasons for using talc in genital hygiene, the risk benefit decision is not complex. Appropriate warnings should be provided to women about the potential risks of regular use of talc in the genital area.

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Exhibit 38

Perineal Talc Exposure and Subsequent Epithelial Ovarian Cancer: A Case-Control Study

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***Objective*: To evaluate the role of talcum powder use as a risk factor for the development of epithelial ovarian cancer.**

***Methods*: In a case-control study, 499 patients with epithelial ovarian cancer were frequency matched for age at diagnosis (±5 years) with a control population of 755 patients. The odds ratio (OR) for the development of epithelial ovarian cancer was estimated using logistic regression analysis with adjustment for age at diagnosis, parity, oral contraceptive use, smoking history, family history of epithelial ovarian cancer, age at menarche, menopausal status, income, education, geographic location, history of tubal ligation, and previous hysterectomy.**

***Results*: Two hundred twenty-one of 462 patients (47.8%) in the study population and 311 of 693 patients (44.9%) in the control population had ever used talcum powder (OR 0.92; 95% confidence interval [CI] 0.24, 3.62). A significant association between duration of talc use and development of epithelial ovarian cancer was not demonstrable for 1–9 years (OR 0.9; 95% CI 0.6, 1.5), for 10–19 years (OR 1.4; 95% CI 0.9, 2.2), or for more than 20 years (OR 0.9; 95% CI 0.6, 1.2). To eliminate the possible confounding variable of surgery for the management of ovarian cancer, we omitted 135 patients in the study population who underwent hysterectomy within 5 years of the diagnosis of ovarian cancer. Within this subgroup of patients, tubal ligation or hysterectomy among talc users still failed to demonstrate an increased risk for the development of ovarian cancer (OR 0.9; 95% CI 0.4, 2.2).**

***Conclusion*: A significant association between the use of talcum powder and the risk of developing epithelial ovarian cancer is not demonstrable, even with prolonged exposure. (Obstet Gynecol 1999;93:372–6. © 1999 by The American College of Obstetricians and Gynecologists.)**

Transvaginal exposure to talcum powder has been proposed as a risk factor for the development of epithelial ovarian cancer, not only because of the chemical similarities between talcum powder and asbestos, but because the two chemical substances are often found together in mineral deposits. In 1967, the seminal work by Graham and Graham[1] demonstrated that intraperitoneal application of asbestos in guinea pigs and rabbits resulted in ovarian epithelial hyperplasia comparable to the histologic changes in early epithelial ovarian tumors observed in women. In 1971, Henderson et al[2] examined the ovarian tissue of 13 patients with epithelial ovarian cancer and reported that 75% of these tumors had talc particles embedded in the tissue. To further support the theory that retrograde flow of talc particles (after direct perineal application of talc) may result in deposition of particles on the ovary, Heller et al[3] analyzed the ovaries of 24 women who underwent oophorectomies for benign ovarian neoplasms. Talcum powder was identified in all 24 patients. Twelve patients reported frequent perineal talc applications, but the remaining 12 denied ever using talc. Although histologic data appear to support the hypothesis that talcum powder applied to the perineum may migrate through retrograde flow, the role of talc as a risk factor for the development of ovarian cancer remains controversial.

The disparity among conclusions regarding the possible association between the use of talcum powder and the risk of ovarian cancer may lie in the fact that not all studies adjusted for the integrity of the genital tract. The integrity of a patient's genital tract is defined as intact when she has not undergone any of the following surgical procedures: salpingo-oophorectomy, bilateral tubal ligation, or hysterectomy (abdominal or vaginal). The purpose of the current study was to evaluate the use of talcum powder as a risk factor for the development of ovarian cancer in an analysis that includes the duration of use and the integrity of the genital tract.

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Materials and Methods

The application of talcum powder to the genital region among 499 patients with epithelial ovarian cancer treated at the Roswell Park Cancer Institute from October 1982 through October 1995 was compared with the application of talcum powder to the genital region among 755 female patients treated for nongynecologic malignancies during the same period. The case and control populations were frequency matched for age at diagnosis (±5 years). Information regarding all patients was extracted from a database compiled from a self-administered questionnaire provided to the patients as a part of the enrollment process at our institute since 1953. The current document, in use since 1982, contains 44 items that pertain to the women's medical and social histories, including parity, menstrual history, use of exogenous hormones, contraceptive history, and personal hygiene. Additional information (besides the 44 items) regarding the patient's medical, social, family, dietary, and occupational histories is available and can be evaluated as potential confounding variables.

The diagnosis of each patient in the study population and in the control population was determined by reference to the Roswell Park Tumor Registry. Patients in the study population were coded with the International Classification of Diseases for Oncology, 1st ed. (ICD-O) code 8010/3 C56.9. The control population was randomly selected from the Roswell Park Tumor Registry. The pool of eligible patients was large enough to select 1.5 control patients for each patient in the study population. The control population consisted of 326 patients (43.3%) with colorectal cancer (ICD-O 8140/3 C16.0–C16.9), 23 patients (3.0%) with stomach cancer (ICD-O 8140/3 C16.0–C16.9), 11 patients (1.5%) with malignancy of the small intestine (ICD-O 8140/3 C17.0–C17.9), 134 patients (17.7%) with leukemia (ICD-O 9800/3-9940/3 C42.1), and 261 patients (34.5%) with malignancies of the skin (ICD-O C44.0–C44.9).

The odds ratio (OR) for the development of epithelial ovarian cancer was estimated using multiple logistic regression analysis with adjustment for oral contraceptive (OC) use, smoking history, family history of epithelial ovarian cancer, age at menarche, menopausal status, income, education, geographic location, and history of tubal ligation or hysterectomy. Among the study population, ORs were determined also to evaluate an association between histologic subtypes of epithelial ovarian cancer and the use of talcum powder, the duration of use of talcum powder and the risk for the development of ovarian cancer, and interruption of the continuity of the reproductive tract by either tubal ligation or hysterectomy and the risk of epithelial ovarian cancer. Data were analyzed using SPSS for Windows, Advanced Professional Release 7.5-1997 (SPSS Inc., Chicago, IL).

Table 1. Patient Characteristics

Characteristic	Cases (n = 499)	Controls (n = 755)	P
Mean age (y)	54.9	54.9	NS
Age at menarche (y)	12.8	12.8	NS
Age at menopause (y)	45.2	45.7	NS
Family history of ovarian cancer			
No	460 (92.2%)	735 (97.4%)	<.001
Yes	39 (7.8%)	20 (2.6%)	
Location			
Erie and Niagara counties	165 (33.3%)	392 (51.8%)	<.001
All others	331 (66.6%)	354 (46.9%)	
Unknown	3 (0.1%)	9 (1.3%)	
Parity			
0	105 (21.0%)	112 (14.8%)	.02
1–2	74 (14.8%)	107 (14.1%)	
3–4	200 (40.0%)	350 (46.4%)	
≥5	120 (24.0%)	186 (24.6%)	
Oral contraceptive use			
No	346 (69.3%)	507 (67.2%)	NS
Yes	148 (29.7%)	236 (31.2%)	
Unknown	5 (1.0%)	12 (1.6%)	
Race			
Black	6 (1.2%)	21 (2.8%)	NS
White	488 (97.8%)	732 (97.0%)	
Other	5 (1.0%)	2 (0.2%)	
Education			
Up to high school	262 (52.5%)	439 (58.2%)	.039
College	237 (47.5%)	316 (41.8%)	
Income			
<$16,000	175 (35.1%)	314 (41.7%)	.013
$16,000–$24,999	133 (26.7%)	157 (20.8%)	
≥$25,000	191 (38.2%)	272 (37.5%)	

NS = not significant.

Results

Characteristics of both the cases and controls are outlined in Table 1. A significantly (P = .039) greater proportion of patients in the study population attended college as compared with the control population, and a significantly (P = .013) greater proportion of patients in the control population had an annual income of less than $16,000. Furthermore, a significantly (P < .001) greater proportion of patients in the study population (7.8%) had a family history of ovarian cancer than did patients in the control population (2.6%). A significantly (P < .001) greater proportion of patients in the control population lived in the counties immediately surrounding our facility compared with patients in the study population. Patients in the control population had significantly (P = .02) more children than did patients in the study population.

Information regarding the use of talc was retrievable

Table 2. Talc Use by Site: Odds Ratios and 95% Confidence Intervals

Site	Controls	Cases	OR* (95% CI)
Never used	382 (55.1%)	241 (52.2%)	1.0
Sanitary napkin	20 (2.9%)	13 (2.8%)	0.9 (0.4, 2.0)
Genital or thigh area	223 (32.2%)	157 (34.0%)	1.0 (0.8, 1.3)
Both	68 (9.8%)	51 (11.0%)	1.1 (0.7, 1.7)

OR = odds ratio; CI = confidence interval.
* Adjusted for parity, oral contraceptive use, smoking history, family history of epithelial ovarian cancer, age at menarche, menopausal status, income, education, geographic location, and history of tubal ligation or hysterectomy.

from the questionnaires of 462 patients in the study population and 693 patients in the control population. Thirty-seven patients in the study population and 62 patients in the control population failed to respond to the question regarding talc use. Among the cases, 47.8% (221 of 462) had ever used talc; 44.9% (311 of 693) of the controls had ever used talc ($P = .323$). Application of talc by site was similar in both groups: 3% of cases and controls applied talc on sanitary napkins, 34% of cases and 32.2% of controls applied talc on the genital or thigh area, and 11% of cases and 9.8% of controls applied talc to both sanitary napkins and the genital or thigh area. The adjusted OR did not demonstrate any increased risk for the development of ovarian cancer attributable to the method of talc application (Table 2).

Thirty-two patients from the case population and 39 patients from the control population did not recall the duration of talc use (Table 3). The mean duration of use was 22 years among patients in the control population and 21 years among patients in the study population. Both groups of women had used talc for a comparable period of time: 9.1% of cases and 9.3% of controls used talc for 1–9 years; 11.4% of cases and 7.6% of controls used talc for 10–19 years; and 23.5% of cases and 24.6% of controls used talc for more than 20 years. Among the study population, a significant association between the duration of talc use and the development of epithelial ovarian cancer was not demonstrable for 1–9 years (OR 0.9; 95% CI 0.6, 1.5), 10–19 years (OR 1.4; 95% CI 0.9, 2.2), or more than 20 years (OR 0.9; 95% CI 0.6, 1.2).

Table 3. Duration of Talc Use: Odds Ratios and 95% Confidence Intervals

Duration (y)	Controls*	Cases†	OR‡ (95% CI)
None	382 (58.4%)	241 (56.0%)	1.0
1–9	61 (9.3%)	39 (9.1%)	0.9 (0.6, 1.5)
10–19	50 (7.6%)	49 (11.4%)	1.4 (0.9, 2.2)
≥20	161 (24.6%)	101 (23.5%)	0.9 (0.6, 1.2)

Abbreviations as in Table 2.
* Thirty-nine patients did not recall duration of use.
† Thirty-two patients did not recall duration of use.
‡ Adjusted for factors stated in Table 2.

Among patients in the study population who had ever applied talcum powder to the perineum or to sanitary napkins, 136 patients (61.6%) had papillary serous cystadenocarcinoma, 21 patients (9.5%) had endometrioid carcinoma, 11 patients (5.0%) had mucinous adenocarcinoma, 12 patients (5.4%) had clear cell adenocarcinoma, and 41 patients (18.6%) had undifferentiated carcinoma. A significant association between the use of talcum powder and a specific histologic subtype of epithelial ovarian cancer was not demonstrable for serous cystadenocarcinoma (OR 1.2; 95% CI 0.7, 2.1), endometrioid carcinoma (OR 1.4; 95% CI 0.7, 2.7), mucinous adenocarcinoma (OR 1.5; 95% CI 0.6, 4.0), clear cell adenocarcinoma (OR 1.6; 95% CI 0.6, 4.3), or undifferentiated carcinoma (OR 1.0; 95% CI 0.6, 1.6).

To assess the impact of surgical interruption of the genital tract as a possible confounding variable, we evaluated the association between ovarian cancer and the use of talcum powder among patients in the study population who had not undergone any interruption of the genital tract compared with those in the study population who had undergone tubal ligation or hysterectomy. Among the cases, 267 patients (53.5%) had not had tubal ligation or hysterectomy, whereas 45.3% (226 patients) had undergone previously tubal ligation or hysterectomy. Of these 226 patients, 59.7% (135 patients) had a hysterectomy within 5 years of being diagnosed with ovarian cancer. Six women in the study group did not answer the question regarding history of tubal ligation or hysterectomy. There was no significant difference in the risk of developing ovarian cancer among patients in the study population with no history of genital tract interruption (OR 1.2; 95% CI 0.8, 1.6) and those with a history of tubal ligation or hysterectomy (OR 0.8; 95% CI 0.5, 1.2). To eliminate the possible confounding variable of surgery for the management of ovarian cancer, we excluded the 135 patients in the study population who had undergone hysterectomy within 5 years of the diagnosis of ovarian cancer. Within this subgroup of patients, tubal ligation or hysterectomy among talc users still failed to demonstrate that the use of talc significantly increased the risk of ovarian cancer (OR 0.9; 95% CI 0.4, 2.2) (Table 4). Multiple logistic regression analysis adjusted for age at diagnosis, parity, OC use, smoking history, family history of epithelial ovarian cancer, age at menarche, menopausal status, income, education, geographic location, and history of tubal ligation or hysterectomy failed to demonstrate any significant association between talc use and the development of ovarian cancer (OR 0.92; 95% CI 0.24, 3.62).

Table 4. Talc Users: Genital Tract Interruption and Risk of Ovarian Cancer

Operation	Talc use		OR* (95% CI)
	No	Yes	
No history of genital tract interruption			1.2 (0.8, 1.6)
Cases	130	121	
Controls	251	182	
History of tubal ligation or hysterectomy			0.8 (0.5, 1.2)
Cases	111	100	
Controls	131	130	
History of hysterectomy			0.9 (0.4, 2.2)
Cases	60	65	
Controls	14	21	

Abbreviations as in Table 2.
* Adjusted for factors stated in Table 2.

Discussion

The current study fails to demonstrate an association between the use of perineal talcum powder and a significant increase in the risk of epithelial ovarian cancer. These findings are at variance with a meta-analytic report by Gross and Berg,[4] which demonstrated a modest increase in the risk of epithelial ovarian cancer among patients who had ever used talc. In an analysis of ten epidemiologic studies, Gross and Berg[4] calculated an adjusted OR of 1.29 (95% CI 1.02, 1.63). Similarly, in a population-based case-control study, Harlow et al[5] noted an increased risk (OR 1.57; 95% CI 1.0, 2.1) for the development of ovarian cancer among patients who had ever used talcum powder. This study, however, failed to adjust for OC use and family history of ovarian cancer. Moreover, 28% of the patients in that study population[5] had borderline ovarian tumors; many investigators believe that this histopathologic entity differs substantially in pathogenesis and clinical course from invasive epithelial ovarian cancer. Despite these limitations, Harlow et al[5] performed a meta-analytic calculation that described a modest association between the development of ovarian cancer and perineal talc use (crude OR 1.3; 95% CI 1.1, 1.6).

The results of the current study also differ from those reported by Cramer et al.[6] These researchers reported a near doubling of risk for the development of epithelial ovarian cancer among talc users (relative risk [RR] 1.92; 95% CI 1.27, 2.89). However, calculation of risk in this study was adjusted only for parity and menopausal status. In a population-based case-control study by Cook et al[7] that reported an adjusted RR of 1.5 (95% CI 1.1, 2.0), these researchers failed to demonstrate a trend in the OR with an increasing number of perineal applications. Important differences exist in the design of our study and that of Cook et al.[7] Failure to adjust for a family history of ovarian cancer is a potential limitation in the study by Cook et al[7] because 26.9% of patients in the study population and 52.1% of the control population were less than 44 years of age (women diagnosed with ovarian cancer before the age of 45 may have a strong genetic predisposition for this disease). Furthermore, in the study by Cook et al,[7] 79 patients (25.2%) in the study population were diagnosed with borderline epithelial ovarian tumor, a histologic entity that may have a different clinical course than invasive epithelial ovarian cancer.

The results of the current report do, however, support the conclusions of three hospital-based, case-control studies that failed to establish a significant association between the use of talc and an increased risk of epithelial ovarian cancer: Booth et al[8] (RR 1.2; 95% CI 0.92, 1.8), Rosenblatt et al[9] (RR 0.8; 95% CI 0.27, 2.63), and Tzonou et al[10] (RR 1.05; 95% CI 0.38, 3.98). In a letter to the editor, Hartge et al (Hartge P, Hoover R, Lesher LP, McGowan L. Talc and ovarian cancer. JAMA 1983;250: 1844) estimated the RR of ovarian cancer among talc users to be 0.7 (95% CI 0.4, 1.1). Additionally, in a collaborative review of hospital- and population-based case-control studies, Whittemore et al[11] failed to confirm a significantly altered risk of epithelial ovarian cancer (RR 1.4; 95% CI 0.98, 1.89) among patients who had applied talcum powder to the perineum.

Duration of exposure and the integrity of the female genital tract are crucial issues in the role of talc exposure as a risk factor for the development of epithelial ovarian cancer. Harlow et al[5] argued that a 70% increase in the risk of ovarian cancer was evident among patients whose exposure to talc exceeded 10,000 applications (equivalent to 30 years of exposure) despite an intact genital tract and normal cyclic ovarian function. These calculations, although intriguing, should be interpreted with caution because they are based on a population of 38 patients, and a test for trend fails to achieve statistical significance ($P = .77$). Whittemore et al[11] observed a similar lack of significance for a trend in the duration of exposure. These researchers demonstrated no significant association between increased ovarian cancer risk and prolonged talc exposure (RR 1.3; 95% CI 0.88, 1.92). Moreover, when the population described by Whittemore et al[11] was stratified based on surgical sterilization and the use of talc, no significant association was observed between the integrity of the genital tract or talc exposure and an increased risk for the development of ovarian cancer. Data from the current study support the findings of Whittemore et al[11] because they demonstrate no significant increase in the risk of epithelial ovarian cancer among patients whose exposure to perineal talc exceeded 20 years, even in the presence of an intact genital tract.

Some significant differences between the study population and the control population were observed in the current study; these differences are in agreement with several epidemiologic studies that evaluated risk factors for the development of ovarian cancer.[5,12–14] In the current study, patients with ovarian cancer acquired a higher level of education ($P = .039$) and higher income ($P = .013$) than the control population; patients in the control population had more children ($P = .02$) than in the study population. A greater proportion of the study group had a significant ($P < .001$) family history of ovarian cancer and lived remote from our institute. The reason may be that Roswell Park Cancer Institute has a referral center, the Gilda Radner Familial Ovarian Cancer Registry, for patients with an apparent genetic predisposition for the development of ovarian cancer. Overall, adjustment for these confounding variables in a multiple logistic regression model failed to demonstrate any significant association (OR 0.92; 95% CI 0.24, 3.62) between the use of talcum powder and the development of ovarian cancer.

Two potential weaknesses of this study should be addressed. First, as with any retrospective study using data collected from the patient's recall of events, potential ascertainment and recall bias may exist. To diminish ascertainment and recall bias, we asked patients to complete a 13-page questionnaire, which contains items pertaining to their medical, social, dietary, and occupational histories; no particular emphasis is placed on any of the items. Second, condoms and diaphragms are potential sources of talc exposure. The questionnaire asks patients whether they have ever used condoms or diaphragms as contraceptive methods; however, this questionnaire does not ask about the frequency or duration of such usage, whether lubricated or nonlubricated condoms were used, or whether talc was ever applied to the diaphragm. Consequently, the current study is limited to the use of talc on the perineum or sanitary napkins and does not address potential talc exposure from condom or diaphragm use.

The results of the current study fail to support the hypothesis that talcum powder usage is associated with the development of epithelial ovarian cancer, regardless of the duration of use and the integrity of the female genital tract.

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Exhibit 39

Factors Related to Inflammation of the Ovarian Epithelium and Risk of Ovarian Cancer

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Previous epidemiologic observations consistently suggest that suppression of ovulation, tubal ligation, and hysterectomy reduce the risk of ovarian cancer and that perineal talc use increases the risk. We examined these and other risk factors in the context of a new hypothesis: that inflammation may play a role in ovarian cancer risk. Ovulation entails ovarian epithelial inflammation; talc, endometriosis, cysts, and hyperthyroidism may be associated with inflammatory responses of the ovarian epithelium; gynecologic surgery may preclude irritants from reaching the ovaries via ascension from the lower genital tract. We evaluated these risk factors in a population-based case-control study. Cases 20–69 years of age with a recent diagnosis of epithelial ovarian cancer (767) were compared with community controls (1367). We found that a number of reproductive and contraceptive factors that suppress ovulation, including gravidity, breast feeding, and oral contraception, reduced the risk of ovarian cancer. Environmental factors and medical conditions that increased risk included talc use, endometriosis, ovarian cysts, and hyperthyroidism. Gynecologic surgery including hysterectomy and tubal ligation were protective. Tubal ligation afforded a risk reduction even 20 or more years after the surgery. The spectrum of associations provides support for the hypothesis that inflammation may mediate ovarian cancer risk. (Epidemiology 2000;11:111–117)

Keywords: ovarian cancer, endometriosis, oral contraceptives, talc, tubal ligation.

Ovarian cancer is a commonly fatal malignancy for which prevention strategies have been limited, in part, by a lack of understanding of its pathobiology. Factors that have consistently been shown to reduce the risk of ovarian cancer, such as number of pregnancies or live births, contraceptive use, and breast-feeding,[1–5] have been proposed to do so by reducing the number of ovulations or the exposure to high pituitary gonadotrophin levels over a lifetime.[6,7] Nevertheless, the biologic mechanism responsible for other consistently demonstrated protective factors is less well understood; such protective factors include: tubal ligation and hysterectomy,[1,8–18] asbestos[19–21] and talc exposures,[2,22–32] endometriosis,[33–35] and perhaps, pelvic inflammatory disease.[36,37]

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See related editorial on page 97 of this issue.

All of these factors may act by a common pathway, by modulating inflammation of the ovarian epithelium, the cell type from which more than 90% of ovarian cancers arise. Ovulation entails disruption of the ovarian epithelium by the extruded follicle, followed by inflammation and wound repair.[38,39] Asbestos, talc, endometriosis, and pelvic inflammatory disease all initiate marked local inflammation. Tubal ligation and hysterectomy sever the pathway from the lower to the upper genital tract, thereby disallowing inflammatory substances to ascend through the lower genital tract to the upper genital tract, and ultimately to the ovarian epithelium.[17] Inflammation involves rapid cell division, DNA excision and repair, oxidative stress, and high concentrations of cytokines and prostaglandins, all of which are established promoters of mutagenesis.[40–43]

Using data from a population-based case-control study of ovarian cancer, we here examine whether factors that are associated with ovarian epithelial inflammation consistently elevate ovarian cancer risk and whether those that reduce the potential for inflammation are protective.

METHODS

Study Subjects

Cases were women 20 through 69 years of age who had had epithelial ovarian cancer diagnosed within the 6 months before the interview. They were ascertained from 39 hospitals around the Delaware Valley including contiguous counties in eastern Pennsylvania, Southern

New Jersey, and Delaware. Between 1994 and 1998, 2,418 cases of histologically confirmed borderline or invasive epithelial ovarian cancer were identified. After excluding women who were not between the ages of 20–69 (640), resided outside the counties in which referral hospitals were located (342), had a prior diagnosis of ovarian cancer (158), or did not speak English or were mentally incompetent (25),[25] we found 1,253 potentially eligible women; after excluding those who were diagnosed more than 6 months before interview (296), were critically ill or dead (69), or were untraceable (15), we were left with 873 women who had incident cancer and were thus eligible for study. Fourteen physicians did not consent to their patients' participation and 92 women refused to participate, resulting in 767 completed case interviews (61% of potentially eligible cases and 88% of potentially eligible, incident cases).

Controls age 65 or younger were ascertained by random digit dialing. These subjects were frequency-matched by 5-year age groups and three-digit telephone exchange to cases. Of the 14,551 telephone numbers screened, 6,597 were businesses or not in service and 5,640 had no female of eligible age in the household, leaving 2,314 households with potentially eligible participants. Of these, 1,928 (83%) had a potentially eligible woman who was willing to be screened further for eligibility. Upon screening, 291 had no eligible woman resident on the basis of age (5), residence outside of the target counties (11), prior diagnosis of ovarian cancer (9), prior bilateral oophorectomy (187), not speaking English or mental incompetence (22), critical illness or death (6), or being untraceable (51). Of the 1,637 screened and potentially eligible controls, 422 declined to be interviewed and 1,215 (74%) were interviewed. Based on our previous experience of lower response rates among 65+ year olds using a random digit dialing approach, we ascertained controls 65–69 years of age through Health Care Financing Administration (HCFA) lists. Four hundred twenty-three women were initially identified, and were frequency-matched to cases by country of residence and age group. One hundred sixty were ineligible for the reasons given above. Of the 263 potentially eligible from HCFA lists, 111 refused to participate and 152 (58%) were interviewed. Therefore, of the total of 1,900 eligible potential controls, 1,367 (72%) are included in these analyses. Age was similarly distributed for cases and controls. Overall, 4% of subjects were age <30, 13% were 30–39, 29% were 40–49, 30% were 50–59, and 24% were 60–69.

Cases included 616 invasive epithelial ovarian cancers and 151 borderline epithelial ovarian tumors. Central pathologic review was conducted on a random sample of 120 cases. The reference pathologist agreed with the original pathologic review for invasiveness in 95% of cases and for cell type in 82% of cases. The original pathologic diagnosis was then used for all cases.

Data Collection

Standardized 1.5-hour interviews were conducted in the homes of participating women by trained interviewers. A life calendar, marked by important happenings that participants recalled during their lives, was used to enhance memory of distant events. On the calendar was coded sexual activity, contraceptives used, and reproductive events for every month from sexual debut until a reference date, defined as the date 6 months before the interview (for both cases and controls).

All pregnancies, their length and outcome as well as the length of breast-feeding, were recorded on the life calendar. The type and length of each contraceptive use was recorded. Tubal ligation, hysterectomy, and ovarian operations were detailed including the time, abdominal *vs* vaginal approach, and procedures done to the ovaries. Menstrual onset, regularity, discomfort, and cessation were recorded. This included questions that asked, "How long did it take before your periods started coming about once a month (without the use of birth control pills)?" and "during your 20's and 30's, and when you were not pregnant, nursing or taking birth control pills/shots/implants, did you ever miss three or more menstrual periods in a row?" The two variables derived from these questions were titled "time to regular menstrual cycles" and "ever miss ≥3 menses." Women were also asked about a series of medical conditions that may be related to pelvic inflammation including ovarian cysts, pelvic inflammatory disease, thyroid disease, and endometriosis. Finally, women were asked about talc use. The question was, "As an adult and prior to [reference date] did you ever use talc, baby or deodorizing powder, at least once per month for 6 or more months on your: 1) feet, arms, or breasts, but not the genital or rectal areas? 2) genital or rectal area? 3) on your sanitary napkins? 4) on your underwear? 5) on your diaphragm or cervical cap?" They were then asked whether they had a male sexual partner(s) for more than a year who regularly used such products on his genital area or underwear. The duration of use of talc for each of these modes of use was also queried.

Statistical Analysis

Because matching was based on frequencies for only two broad criteria, age within 5-year intervals and three-digit telephone exchanges (or county of residence), we did not preserve the "match" in the analyses. We adjusted odds ratios for age and gravidity (each as continuous variables), race (white/black/other), history of ovarian cancer in any first degree relative (yes/no), oral contraceptive use (yes/no), tubal ligation (yes/no), hysterectomy (yes/no), and breast-feeding (yes/no) by unconditional logistic regression analysis.[44]

RESULTS

We first examined reproductive and contraceptive factors that would be expected to affect ovulation and/or endogenous steroid hormones. Pregnancies and breast-feeding were protective for ovarian cancer, with a 50% risk reduction associated with the first pregnancy and a modest additional lowering of risk for pregnancies beyond the first. Breast-feeding reduced risk after at least

TABLE 1. Selected Characteristics Related to Reproductive History and Ovarian Cancer

Variable	Cases (767)	Controls (1367)	Crude OR	95% CI	Adjusted OR*	95% CI
Pregnancies						
0	176	127	1.0		1.0	
1	107	140	0.5	0.4–0.8	0.6	0.4–0.9
2	177	346	0.4	0.3–0.5	0.4	0.3–0.6
3	142	308	0.3	0.2–0.4	0.4	0.3–0.5
4	70	194	0.3	0.2–0.4	0.3	0.2–0.4
≥5	95	252	0.3	0.2–0.4	0.3	0.2–0.4
Oral contraceptive duration (years)						
Never	341	426	1.0		1.0	
<1	141	266	0.7	0.5–0.8	0.8	0.6–1.0
1–4	162	362	0.6	0.4–0.7	0.7	0.6–1.0
5–9	88	189	0.6	0.4–0.8	0.7	0.5–1.0
≥10	32	120	0.3	0.2–0.5	0.4	0.2–0.6
Breast-fed (months)†						
Never	299	577	1.0		1.0	
1–5	117	259	0.9	0.7–1.1	0.9	0.7–1.2
6–11	46	119	0.9	0.5–1.1	0.9	0.6–1.3
12–23	40	124	0.6	0.4–0.9	0.7	0.5–1.1
≥24	29	111	0.5	0.3–0.8	0.6	0.4–1.0
Age at menarche (years)						
≤11	171	316	1.0		1.0	
12	205	382	1.0	0.7–1.3	1.0	0.8–1.3
13	203	341	1.0	0.8–1.3	1.0	0.8–1.4
≥14	188	328	1.0	0.8–1.3	1.0	0.8–1.3
Age at natural menopause (years)‡						
<45	108	186	1.0		1.0	
45–49	122	183	1.1	0.8–1.6	0.9	0.6–1.4
50–52	84	123	1.2	0.8–1.7	0.9	0.6–1.4
≥53	53	74	1.2	0.8–1.9	1.0	0.6–1.6
Length of menstrual cycles (days)						
26–34	599	1044	1.0		1.0	
≤25	82	139	1.0	0.8–1.4	1.1	0.8–1.5
≥35	25	54	0.8	0.5–1.3	0.8	0.5–1.4
Never regular	47	93	0.9	0.6–1.3	1.0	0.7–1.4
Time to regular menstrual cycles (months)						
1	480	898	1.0		1.0	
2–6	143	208	1.3	1.0–1.6	1.3	1.0–1.7
7–12	53	102	1.0	0.7–1.4	1.1	0.8–1.6
≥13	65	125	1.0	0.7–1.3	1.0	0.7–1.5
Ever miss ≥3 menses						
No	719	1293	1.0		1.0	
Yes	41	64	1.2	0.8–1.7	1.2	0.8–1.8

* Adjusted for age, number of pregnancies, family history of ovarian cancer, race, oral contraceptive use, tubal ligation, hysterectomy, and breast-feeding.
† Among women who had had a live birth.
‡ Excludes women who were pre-menopausal, had had a hysterectomy, or were using hormone replacement therapy prior to the cessation of menses.

24 months of breast-feeding over a lifetime (Table 1). Oral contraception provided a duration-dependent reduction in risk. Neither age at menarche nor age at natural menopause was strongly associated with ovarian cancer risk. Similarly, menstrual patterns had little association with ovarian cancer risk. In particular, never having regular menstrual cycles, which may be a marker of anovulation, did not substantially lower ovarian cancer risk.

We next evaluated environmental factors and medical conditions that might be associated with increased local inflammation (Table 2). Talc use on all areas of the body elevated ovarian cancer risk, even after adjustment for potentially important confounding factors. Similarly, talc use on sanitary napkins and underwear elevated ovarian cancer risk. In contrast, talc use on diaphragms and/or cervical caps and use by the male partner did not appear to alter risk by much. Furthermore, length of use was not clearly related to risk.

Three medical conditions increased ovarian cancer risk in our data: ovarian cysts (adjusted OR 1.3), endometriosis (adjusted OR 1.7), and hyperthyroidism (adjusted OR 1.8). The relation between these medical conditions and ovarian cancer appeared to be relatively specific. We did not, for example, find associations between ovarian cancer and lower genital tract infections that do not cause ovarian inflammation, such as genital warts and herpes simplex infection (data not shown). Nevertheless, pelvic inflammatory disease, which does cause ovarian inflammation, was only modestly associated with ovarian cancer in our data.

Gynecologic surgery, including tubal ligation and hysterectomy, reduced ovarian cancer risk (Table 3). Tubal ligation and tubal ligation in combination with hysterectomy both provided marked protection (odds ratios 0.5 and 0.4). Hysterectomy without tubal ligation was less protective (odds ratio 0.8). To examine the possibility that surveillance bias, that is removal of an unusual appearing ovary destined to become cancer, may have accounted for these relations, we evaluated the association between time since gynecologic surgery and ovarian cancer risk. Although there was a trend toward

TABLE 2. Environmental Factors and Medical Conditions and Ovarian Cancer

Variable	Cases 767	Controls 1367	Crude OR	95% CI	Adjusted OR*	95% CI
Talc use†						
Never	349	728	1.0		1.0	
Feet, etc	335	512	1.4	1.1 –1.6	1.4	1.1–1.6
Genital/rectal	161	219	1.5	1.2 –1.9	1.5	1.1–2.0
Sanitary napkin	77	94	1.7	1.2 –1.9	1.6	1.1–2.3
Underwear	70	100	1.5	1.0 –2.0	1.7	1.2–2.4
Diaphragm/Cerv Cap	10	33	0.6	0.3 –1.3	0.6	0.3–1.2
Male partner	56	126	0.9	0.7 –1.3	1.0	0.7–1.4
Talc use (genital/rectal and feet)						
Never	401	819	1.0		1.0	
<1 year	17	17	2.0	1.0 –4.0	2.0	1.0–4.0
1–4 years	76	101	1.5	1.1 –2.1	1.6	1.1–2.3
5–9 years	40	59	1.4	0.9 –2.1	1.2	0.8–1.9
10+ years	233	371	1.3	1.0 –1.6	1.2	1.0–1.5
Ovarian cysts						
No	604	1131	1.0		1.0	
Yes	154	231	1.2	1.0 –1.6	1.3	1.1–1.7
Thyroid disease						
Never	646	1165	1.0		1.0	
Overactive	30	30	1.8	1.1 –3.0	1.8	1.0–3.0
Underactive	72	138	0.9	0.7 –1.3	0.9	0.6–1.2
Endometriosis						
No	698	1279	1.0		1.0	
Yes	66	85	1.5	1.0 –2.1	1.7	1.2–2.4
Pelvic inflammatory disease						
No	752	1335	1.0		1.0	
Yes	14	27	0.9	0.5 –1.8	1.3	0.6–2.5

* Adjusted for age, number of pregnancies, family history of ovarian cancer, race, oral contraceptive use, tubal ligation, hysterectomy, and breast-feeding.
† Subjects may have used talc on more than one area of the body so numbers add to more than 767 cases and 1,367 controls.

less protection with a longer period of time since tubal ligation, risk reduction was still afforded to women with that surgery 20 or more years earlier. Abdominal hysterectomy, which affords a direct view of the abdominal cavity, was not more protective than vaginal hysterectomy, which does not afford a direct view, making it unlikely that removal of an abnormal-appearing ovary at surgery accounted for the protection afforded by gynecologic operations. Laparoscopy for reasons other than tubal ligation or ovarian operations, which would also allow a surgeon to inspect the ovaries and remove any

TABLE 3. Gynecologic Surgery and Ovarian Cancer

Variable	Cases (767)	Controls (1367)	Crude OR	95% CI	Adjusted OR*	95% CI
Neither	570	797	1.0			
Tubal ligation	117	388	0.4	0.3–0.5	0.5	0.4–0.7
Hysterectomy	67	118	0.8	0.6–1.1	0.8	0.6–1.1
Both	13	63	0.3	0.2–0.5	0.4	0.2–0.8
Time since tubal ligation (years)						
Never	637	915	1.0			
<2	3	20	0.2	0.1–0.7	0.3	0.1–1.1
2–9	21	80	0.4	0.2–0.6	0.6	0.4–1.0
10–19	53	194	0.4	0.3–0.5	0.5	0.3–0.7
≥20	53	155	0.5	0.4–0.7	0.6	0.4–0.9
Time since hysterectomy						
Never	687	1186	1.0			
<2	2	4	0.9	0.2–4.7	1.0	0.2–6.3
2–9	14	39	0.6	0.3–1.2	0.9	0.5–1.6
10–19	25	68	0.6	0.4–1.0	0.7	0.4–1.2
≥20	39	69	1.0	0.7–1.5	0.8	0.5–1.3
Type of hysterectomy						
Never	687	1186	1.0			
Abdominal	60	113	0.9	0.7–1.3	0.9	0.6–1.3
Vaginal	20	66	0.5	0.3–0.9	0.6	0.3–1.0
Laparoscopy						
Never	711	1264	1.0			
Ever	55	102	1.0	0.7–1.3	1.0	0.7–1.5

* Adjusted for age, number of pregnancies, family history of ovarian cancer, race, oral contraceptive use, and breast-feeding.

that were abnormal, did not affect the risk of ovarian cancer substantially.

DISCUSSION

These analyses are generally, albeit not completely, consistent with the hypothesis that inflammation at the site of the ovarian epithelium is associated with ovarian cancer risk. Our results confirmed the findings of a number of previous studies showing that pregnancies, oral contraceptive use, and prolonged breast feeding, reduce the risk of ovarian cancer.[1–5] All of these factors mark a diminution in number of ovulations and a reduction in pituitary gonadotrophin levels (during breastfeeding, LH levels are suppressed, but FSH levels are not).[45] We did not find, however, that markers of the length of the reproductive window, such as age at menarche, age at natural menopause, and the regularity of menses, were substantially related to ovarian cancer risk. These factors have been inconsistently and/or weakly related to risk in previous studies,[46] perhaps because they inaccurately reflect ovulatory function. The initiation and cessation of menses do not correlate well with the initiation and cessation of ovulation, whereas pregnancy and oral contraceptive use more validly reflect termination of ovulation.[5,47,48]

We found that several factors, which may reflect an inflammatory process at the site of the ovarian epithelium, increase the risk for ovarian cancer. Talc use applied to any part of the body or to sanitary napkins or underwear was related to ovarian cancer risk. These observations are consistent with findings from several previous studies.[2,22–32] Of the 12 epidemiologic studies that we identified that have evaluated the use of talc in relations to ovarian cancer,[2,22–32] 10 have reported at least some elevation in cancer risk among women. In the most extensive and focused analysis to date, Cook *et al.*[22] analyzed data from 313 cases of ovarian cancer and 422 controls regarding recalled use of talc products.[22] Use in the perineal area, powder on sanitary napkins, and genital deodorant spraying were all associated with elevated ovarian cancer risk, whereas, as in our study, use on a diaphragm was not. We found, however, that a small number of women used powder on their diaphragm, thereby limiting the interpretation of these data. The lack of specificity for the body part on which talc was used may be related to the fact that small particles of talc often become airborne during use so that a broader area of the body may be exposed than that to which the talc was directly applied. Some previous studies have found dose-response or duration-response relations between talc use and ovarian cancer,[24,27] whereas others have not.[2,22,31] The reasons for this are unclear.

Endometriosis, the presence of endometrial tissue outside the endometrium, causes a marked local inflammatory reaction. It has been linked to ovarian cancer in a variety of epidemiologic and clinical studies.[33–35,49] Brinton *et al.* assessed cancer outcomes among over 20,000 women hospitalized for endometriosis in Sweden after a mean of 11 years of follow-up.[49] Ovarian cancer risk was elevated as much as fourfold for women whose endometriosis arose in the ovaries. A series of clinical studies have also demonstrated ovarian malignancies arising from ovarian endometriosis, that is, from endometriosis that resides in the ovarian epithelium.[33–35]

Finally, although the link between simple ovarian cysts and ovarian cancer is not clearly established, complex cysts, which may have neoplastic or physiologic components and involve marked local inflammation, may be precursor lesions for ovarian cancer. In our study, we could not distinguish between types of cysts inasmuch as our data were based on self-report. A previous case-control study, which also did not distinguish between pathologic sub-types, found that ovarian cysts were associated with a 12-fold increased risk of ovarian cancer.[36]

The association between hyperthyroidism and ovarian cancer is a novel observation, to our knowledge, and may provide an important clue to our hypothesis. The most common cause of hyperthyroidism is autoimmune inflammation of the thyroid gland.[50] Autoimmune hyperthyroidism, including Graves' disease and Hashimoto's thyroiditis, involve both humorally mediated and cellular immune responses directed against thyroid microsomes, thyroglobulin, and thyroid antigen.[51] They are much more common in women than in men. These diseases run in families in which there are higher rates of other autoimmune disorders, including insulin-dependent diabetes mellitus, rheumatoid arthritis, and systemic lupus erythematosus. Autoimmune diseases in general, and autoimmune thyroid disorder in particular, are systemic, causing inflammation and infiltration beyond the thyroid (*eg* infiltrative ophthalmopathy and dermopathy in Graves disease). There are a variety of reproductive manifestations of hyperthyroidism that have been attributed to thyroid hormonal influences on steroid hormone metabolism.[52] Fertility is impaired, however, even in women with ovulatory cycles, and it is possible that a local inflammatory process may be involved, although to our knowledge, this has never been studied. It would have been interesting to have examined the relations between other autoimmune diseases and ovarian cancer, but, we did not ask about other autoimmune diseases in this study.

We found only a modest elevation in risk associated with pelvic inflammatory disease, whereas the association has been more clearly shown in some previous studies.[36,37] In our study, however, only about 2% of cases and of controls reported previous pelvic inflammatory disease and the power to detect the association was limited. In contrast, national surveys have suggested that 10% or more of American women report this condition during their reproductive lives.[53] This discrepancy suggests the possibility that our study participants substantially under-reported pelvic inflammatory disease (which, because of its links to sexually transmitted diseases, is more socially sensitive than other exposures queried in this study) and may account for the lack of a clearer study finding.

Our data and data from previous studies[1,8–18] suggest that gynecologic surgery, in particular tubal ligation, greatly reduce the risk of ovarian cancer. The following observations, taken as a whole, argue against a surveillance bias whereby abnormal ovaries are removed at the time of surgery: (1) ovarian cancer risk is reduced 20 or more years after tubal ligation; (2) vaginal hysterectomy, which is accomplished without observation of the ovaries, is not less protective than abdominal hysterectomy, wherein ovaries are observed; and (3) laparoscopy does not lower risk. We believe that the protection afforded by gynecologic surgery, and, in particular by tubal ligation, which is generally accomplished earlier in life, is mediated by disrupting the connection between the ovaries and the rest of the genital tract structures.[17] Substances that may cause lower genital tract inflammation, such as talc, can travel up an open genital tract, but with tubal ligation or hysterectomy, that pathway is cut-off, thereby reducing the risk of environmentally mediated inflammation.

Inflammation by its nature produces toxic oxidants meant to kill pathogens. These oxidants cause direct damage to DNA, and it has been proposed that oxidative damage to DNA underlies all mutagenesis. In particular, Ames *et al.* argue that damage to critical tumor suppressor genes, particularly in the situation of rapid cell division, as is found in inflammation, contributes to cancer development.[40] Furthermore, bioactive substances, such as cytokines, growth factors, and prostaglandins, which are part of the inflammatory process, may play a role in ovarian mutagenesis.[41–43] Disregulated cytokines may lead to ovarian neoplasm progression, and overexpression of prostaglandins, which is more common in tumor cells, increases tumor invasiveness.[54]

A limitation of our study was the somewhat low participation rates among cases and controls. For cases, this factor was strongly influenced by whether women with prevalent ovarian cancer (diagnosed >6 months before interview) were included in the denominator when calculating response rates. In our design, we excluded such women to avoid survival bias. Excluding them from the denominator resulted in an 88% response rate; however, to the extent that exposures may differ in women with incident ovarian cancer from those with ovarian cancer overall, we report the 61% response rate with them included in the denominator. Another limitation of our study is the potential for recall bias, which is a concern with any case-control study. In our study, however, recall bias is unlikely to explain factors that appear to be protective, which was the case for many of the associations found. As well, medical risk factors were not universally associated with elevated risk, but, instead the associated factors appeared to be specifically those causing inflammation. A final limitation is that many of our effect sizes were modest.

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Exhibit 40

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PERINEAL TALC EXPOSURE AND EPITHELIAL OVARIAN CANCER RISK IN THE CENTRAL VALLEY OF CALIFORNIA

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Perineal talc use has been suggested as a possible risk factor for ovarian cancer based on its structural similarity to asbestos, a known human carcinogen. A population-based epidemiologic case-control study of epithelial ovarian cancer (EOC) was conducted in 22 counties of Central California that comprise the reporting area for 2 regional cancer registries. Telephone interviews were conducted with 256 cases diagnosed in the years 2000–2001 and 1,122 controls frequency-matched on age and ethnicity. The interview obtained information on demographic factors, menstrual and reproductive experience, exogenous hormone use, surgical history and family history of cancer. Questions on perineal talc use included frequency of use, duration of use and specific years when talc was used. Multivariate-adjusted odds ratio (OR) and 95% confidence intervals (CI) were derived from unconditional logistic regression. The OR for ever use of talc was 1.37 (CI = 1.02–1.85) compared to never users. However, no dose response association was found. Tubal ligation (TL) modified the effect of talc on EOC such that women with TL had an OR of 0.88 (CI = 0.46–1.68) associated with perineal talc use, whereas women with no TL had an OR of 1.54 (CI = 1.10–2.16). Talc use and EOC risk was highest in women with serous invasive tumors (OR = 1.77; CI = 1.12–2.81). This study provides some support for the hypothesis that perineal talc use is associated with an increased risk of EOC.

Key words: *epidemiology; gynecologic cancer; risk factors*

Interest in the relationship between talcum powder and ovarian cancer risk is based on certain physical properties of talcum powder, including the fact that talc is mineralogically similar to asbestos and that talcum powder manufactured before 1973 may have been contaminated with asbestos.[1] In animal studies, talc and other similar substances have been demonstrated to migrate from the vagina through the peritoneal cavity to the ovaries.[2] Henderson *et al.*[3] also observed that particles with the appearance of talc were more prevalent in ovarian tumors than in normal ovarian tissue. Several epidemiologic studies have investigated perineal use of talcum powder as a potential risk factor for ovarian cancer and most have found elevations in risk, although there has been a large range in the risk estimates, from 1.1 to 3.9.[4] Collectively, these studies point to a possible etiologic role of talc in ovarian cancer via an inflammatory process at the site of the ovarian epithelium,[5] although recall bias may play a role in retrospective studies.[4] Inflammation produces oxidants that are thought to damage DNA and Ames *et al.*[6] argue that damage to tumor suppressor genes caused by the inflammatory process leads to carcinogenesis. Chronic inflammation may also result in deregulated cytokine production, which may result in altered cell growth, inhibition of apoptosis and changes in differentiation.[7]

Cramer *et al.*[8] proposed 2 mechanisms that might explain talc carcinogenesis. Talc may stimulate the entrapment of the ovarian surface epithelium, thus mimicking what occurs during ovulation and posing a risk similar to that proposed by incessant ovulation.[9] Alternatively, if talc is present at the time of ovulation, it may become incorporated into the inclusion cyst. It has been suggested that foreign-body exposure may result in granulomas[10] and that pure talc may induce granulomas in open wounds.[11] Granulomas are also associated with persistent acute inflammatory responses.[12]

The role of cornstarch powder on ovarian cancer risk has also been evaluated in epidemiologic research and a recent review concluded that there is no association between this type of powder and increased risk of ovarian cancer.[13] The conclusion was based on a total of 4 case-control studies that elicited information on use of cornstarch in perineal dusting, in which the average odds ratio was 0.62. However, there were only a total of 20 cases of ovarian cancer combined in those studies and 51 control subjects. Cornstarch is also not thought to exert the same toxicologic reaction in human tissue as does talc.[13]

MATERIAL AND METHODS

A population-based epidemiologic case-control study of epithelial ovarian cancer (EOC) was conducted in 22 counties of Central California that comprise the reporting area for 2 regional cancer registries. Geographically, these counties make up the majority of the Central Valley of California, which is the poorest area of the state, with many residents living below the poverty level.[14] Demographically, the Valley is a very ethnically diverse area in which many counties are over 40% Hispanic. Two population-based cancer registries have monitored cancer incidence in the Central Valley of California continuously since 1988: the Cancer Registry of Central California (CRCC) in Fresno and the Cancer Surveillance Program (CSP), Region 3, in Sacramento.[15,16] All newly diagnosed histologically confirmed EOC patients were available for inclusion in this study for the years 2000 and 2001.

Cases were women-identified via a rapid case ascertainment (RCA) procedure as having been diagnosed with EOC (malignant neoplasms of the ovary, ICD-O 3 = C56.9) living in the Central Valley during a 24-month period from 1 January 2000 through 31 December 2001. Tumors were designated as borderline if the behavior code was designated as 1, or if the pathology report described the tumor as borderline, low malignant potential, or atypically proliferating.[17] The borderline classification was limited to serous and mucinous cell types because ICD-O 3 has no morphology code for the borderline classification in the other subtypes and because serous and mucinous tumors make up the majority of borderline tumors.[18] All other tumors were classified as invasive.

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Histologic subtypes were identified by pathologic report or by ICD-O 3 morphology codes. The histologic subtypes included were serous, mucinous, endometrioid, clear cell and other epithelial/unclassified. The latter category included unspecified adenocarcinomas as well as undifferentiated tumors in which a cell type could not be classified histologically. All newly diagnosed EOCs of epithelial origin were identified via RCA methods in which hospital tumor registrars were asked to provide listings of newly diagnosed EOCs within 1 month of diagnosis. A board-certified pathologist reviewed the pathology reports of a sample of cases. Physician consent was obtained by mailing the physician of record a letter and informing him/her that an interview with the patient was planned. If the physician did not respond within a 3-week period, passive consent was assumed. The control group consisted of women 18 years or older selected by random digit dialing (RDD) techniques who were residents of the area, had not been diagnosed with EOC and had at least one intact ovary at the time of the interview. Controls were frequency matched to cases on age and race/ethnicity. The overall data collection period covered a 2-year period, with each respondent being interviewed only once during this period by telephone. Interviews were conducted with both cases and controls on a monthly basis throughout the 2-year period.

All cases and controls were approached via an introductory letter that included a prompt list that described topics the interview questions would address. The Institutional Review Board at the Public Health Institute approved the study protocol. For both case and control groups, letters and prompt lists were sent in either English or Spanish on the letterhead of the principal investigator. Telephone interviews with both case and control respondents were conducted by female professional, trained telephone interviewers in either English or Spanish as preferred by the respondent.

The interview obtained information on demographic factors as well as information pertinent to the respondent's menstrual and reproductive experience, use of exogenous hormones, gynecologic surgical history and family history of cancer. Four questions were asked in regard to the use of talcum powder, including adult use in the genital area, calendar year(s) of use, frequency of use (*i.e.*, daily, several times a week) and total duration of use. The last 2 questions were used to create a variable reflecting—cumulative use by combining frequency (categorically weighted 0–3) and duration (in months) of use.

Age-adjusted odds ratios were calculated using the Mantel-Haenszel method.[19] Multivariate adjusted odds ratios were calculated using unconditional logistic regression.[20] Initially, multivariate models were constructed to include age as a continuous variable and race/ethnicity, duration of use of oral contraceptives, duration of breast-feeding, history of breast or EOC in a first-degree relative, pregnancy history, parity, body mass index (BMI), hysterectomy, tubal ligation and duration of hormone replacement therapy use as categorical variables. However, the Hosmer-Lemshow goodness-of-fit tests revealed that after terms for duration of oral contraceptive use and duration of breast-feeding were added to the models, fit was not improved by the addition of the other variables listed above. Nor were the estimated odds ratios altered by the addition of the several variables listed above. Therefore, in the interest of parsimony, the final models chosen for the analysis included terms for age, race/ethnicity, oral contraceptive use and breast-feeding. Interaction was assessed by comparing stratum-specific odds ratios. If the stratum-specific odds ratios differed by more than 100%, interaction was also assessed by including first-order cross product terms into the logistic model and examining the significance of the interaction coefficient. Tests for trend were conducted for variables that were ordinal in nature by recoding the categories into continuous form and evaluating the Wald statistic associated with the resulting coefficient. Confounding was assessed by examining the differences in the crude, age-adjusted and multivariate-adjusted odds ratios.

RESULTS

The regional cancer registries initially identified a total of 652 cases of confirmed epithelial ovarian cases residing in the 22 county study area diagnosed between 1 January 2000 and 31 December 2001. Seventeen cases were excluded due to speaking a language other than English or Spanish or due to hearing/speech impairment, resulting in 635 cases that met the study criteria. Seventy-six cases died prior to research contact and physicians refused permission to contact for 10 cases. Forty-one cases were too ill to participate in the study and 119 were not contacted due to incorrect telephone numbers or no answer after repeated efforts. Of the remaining 389 cases, 133 refused to participate, resulting in 256 completed interviews. Therefore, the response fraction was 40% among all cases identified. There were no significant differences in age ($p = 0.273$) or level of invasiveness between interviewed and noninterviewed cases. Histologically, interviewed cases were more likely to be of the serous subtype (57.4% for interviewed cases, 45.6% for noninterviewed cases) and less likely to be classified as "other epithelial" (10.5% for interviewed cases; 22% for noninterviewed cases). There was no statistically significant difference between interviewed and noninterviewed cases for the other histologic subtypes. Information on perineal talc use was missing in 7 cases.

Households with eligible women were identified through RDD methods, resulting in 2,327 controls identified and sent an introductory letter with a prompt list. Eighty of these women were later found not to meet the age requirement and 21 were ineligible due to residence outside the study area. Ten controls were excluded due to speaking a language other than English or Spanish. Two hundred fifty-two controls were excluded due to reporting bilateral oophorectomy, resulting in 1,964 controls that met the study criteria. Nineteen controls were too ill to participate and 358 were later found to have moved, changed phone numbers, or failed to answer after repeated efforts. Of the remaining 1,587 contacted controls, 465 refused to participate, resulting in 1,122 completed control interviews for a response fraction of 57% for total identified eligible controls. Information on perineal talc exposure was missing in 17 controls.

Invasive tumors constituted 71.1% of the case series and 28.9% of the tumors were of borderline malignancy. Among non-Hispanic white women, who constituted 74% of the cases, 25.8% were of borderline invasiveness. Overall, 57% of the case series were serous adenocarcinomas, divided 60% and 40% for invasive and borderline, respectively. Mucinous and endometrioid each comprised 14% of the EOC cases. There were slightly more mucinous borderline cases than invasive cases. Clear cell and other/unclassified histologies made up the remaining 5% and 11%, respectively.

The demographic characteristics of all cases and controls and cases and controls stratified by talc exposure are shown in Table I. Matching was successful and cases and controls were similar in age and ethnicity. Controls were less likely to have finished high school but more likely to have an education beyond high school. A somewhat larger proportion of the case series were single (12.5%) compared to the control series (10.0%). Control women were more likely to have been born outside of the United States (16.8%) than were cases (12.9%).

A total of 42.6% of EOC cases reported ever use of talcum powder in the perineal area while 37.1% of control women reported such a history. Case women using talc were slightly older at interview than controls. Women in the oldest age group used talc less than younger women, much more so for control women than case women. White non-Hispanic women were more likely to use talc than their Hispanic counterparts. Talc use was higher in both white non-Hispanic and Hispanic cases compared to controls but this pattern was not seen in the "other" ethnic category. Talc use was also associated with a higher education level. Talc use was higher in both cases and controls with birthplace in the United States.

TABLE I – DESCRIPTIVE CHARACTERISTICS OF EOC CASES AND CONTROLS IN CALIFORNIA'S CENTRAL VALLEY BY TALC EXPOSURE, 2000–2001

Characteristic	Cases		Controls	
	Total[1]	Talc exposure, *n* (%)	Total[1]	Talc exposure, *n* (%)
Number of subjects	249	106 (42.6)	1105	410 (37.1)
Mean age at interview	56.6	56.6	55.0	53.7
Age group (%)				
< 40	20	7 (35.0)	112	43 (38.4)
40–49	66	34 (51.5)	317	121 (38.2)
50–59	57	20 (35.1)	268	113 (42.2)
60–69	56	25 (44.6)	211	82 (38.9)
≥ 70	50	20 (40.0)	197	51 (25.9)
Ethnicity (%)				
White non-Hispanic	187	85 (45.5)	802	317 (39.5)
Hispanic	42	15 (35.7)	201	54 (26.9)
Other	20	6 (30.0)	102	39 (38.2)
Education (%)				
< high school graduate	33	13 (39.4)	208	60 (28.8)
High school graduate	84	34 (40.5)	261	99 (37.9)
> high school graduate	130	59 (45.4)	635	251 (39.5)
Marital status (%)				
Single	32	11 (34.4)	111	40 (36.0)
Married	133	59 (44.4)	670	246 (36.7)
Divorced/separated	40	17 (42.5)	175	77 (44.0)
Widowed	44	19 (43.2)	145	46 (31.7)
Birthplace (%)				
In United States	216	96 (44.4)	919	379 (41.2)
Outside United States	33	10 (30.3)	186	31 (16.7)

[1]Numbers may not add up to total cases and controls due to missing data.

TABLE II – FREQUENCIES, MULTIVARIATE-ADJUSTED ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR PATTERNS OF TALC USE FOR EOC CASES AND CONTROLS, CENTRAL VALLEY OF CALIFORNIA, 2000–2001

Patterns of talc use	Cases (%) (n = 256)[1]	Controls (%) (n = 1,122)[1]	Multivariate-adjusted OR (95% CI)
Talc use			
Never	143 (57.4)	695 (62.9)	1.0
Ever	106 (42.6)	410 (37.1)	1.37 (1.02–1.85)
Frequency of use			
Never	143 (57.4)	695 (63.2)	1.0
Rarely to several times per month	34 (13.7)	138 (12.5)	1.34 (0.87–2.08)
1–3 times per week	31 (12.4)	145 (13.2)	1.16 (0.74–1.81)
4–7 times per week	41 (16.5)	122 (11.1)	1.74 (1.14–2.64)
			Trend p = 0.015
Duration of use			
Never	143 (58.9)	695 (64.2)	1.0 (referent)
≤ 3 years	18 (7.4)	99 (9.2)	1.01 (0.58–1.76)
4–12 years	32 (13.2)	98 (9.1)	1.86 (1.16–2.98)
13–30 years	29 (11.9)	102 (9.4)	1.45 (0.90–2.32)
> 30 years	21 (8.6)	88 (8.1)	1.22 (0.72–2.08)
			Trend p = 0.045
Cumulative use (frequency × duration)			
Never	143 (58.9)	695 (64.4)	1.0 (referent)
First quartile (lowest exposure)	18 (7.4)	95 (8.8)	1.03 (0.59–1.80)
Second quartile	28 (11.5)	95 (8.8)	1.81 (1.10–2.97)
Third quartile	34 (14.0)	107 (9.9)	1.74 (1.11–2.73)
Fourth quartile (highest exposure)	20 (8.2)	88 (8.1)	1.06 (0.62–1.83)
			Trend p = 0.051

Adjusted for age, race/ethnicity, duration of oral contraceptive use and breast feeding. [1]Numbers may not add up to total cases and controls due to missing data.

Ever use of talcum powder in the genital area was associated with a 37% elevation in risk of EOC, which was statistically significant (Table II). Increasing frequency of use was associated with increasing risk such that those women who reported use 4–7 times per week experienced a significant 74% elevation in EOC risk (p for trend = 0.015). However, this was not a monotonic trend in that risk decreased between the second and third categories of use (from 1.34 to 1.16). Duration of use of talcum powder was associated with increased risk, although the pattern was also not clear-cut in that the point estimate peaked among those reporting 4–12 years of use and declined somewhat among those reporting longer duration of use (p for trend = 0.045). Cumulative use also demonstrated an uneven association with risk of EOC in that the point estimates peaked in the second and third quartiles of intensity but declined in the highest quartile of use.

The multivariate adjusted odds ratios were elevated primarily among those with a serous or mucinous invasive tumor and were lower among women with other cell types or with borderline tumors (Table III).

Risk of EOC associated with use of talcum powder was higher and statistically significant in those who reported first using pow-

TABLE III – FREQUENCIES, MULTIVARIATE-ADJUSTED ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR PERINEAL TALC USE AND EOC RISK BY INVASIVENESS AND HISTOLOGIC SUBTYPE, CENTRAL VALLEY OF CALIFORNIA, 2000–2001

Histologic subtype	Cases (%) (n = 256)[1]	Controls (%) (n = 1,122)[1]	Multivariate-adjusted OR (95% CI)
All invasive (n = 182)[1]			
− perineal talc use	98 (55.7)	696 (62.9)	1.0 (referent)
+ perineal talc use	78 (44.3)	410 (37.1)	1.51 (1.07–2.12)
Serous invasive (n = 92)[1]			
− perineal talc use	46 (52.3)	696 (62.9)	1.0 (referent)
+ perineal talc use	42 (47.7)	410 (37.1)	1.77 (1.12–2.81)
Mucinous invasive (n = 16)			
− perineal talc use	6 (37.5)	696 (62.9)	1.0 (referent)
+ perineal talc use	10 (62.5)	410 (37.1)	2.56 (0.89–7.39)
Endometrioid (n = 35)			
− perineal talc use	21 (60.0)	696 (62.9)	1.0 (referent)
+ perineal talc use	14 (40.0)	410 (37.1)	1.28 (0.62–2.62)
Clear cell (n = 12)[1]			
− perineal talc use	8 (72.7)	696 (62.9)	1.0 (referent)
+ perineal talc use	3 (27.3)	410 (37.1)	0.63 (0.15–2.64)
Other epithelial (n = 27)[1]			
− perineal talc use	17 (65.4)	696 (62.9)	1.0 (referent)
+ perineal talc use	9 (34.6)	410 (37.1)	1.06 (0.45–2.48)
All borderline (n = 74)[1]			
− perineal talc use	45 (61.6)	696 (62.9)	1.0 (referent)
+ perineal talc use	28 (38.4)	410 (37.1)	1.09 (0.65–1.83)
Serous borderline (n = 55)[1]			
− perineal talc use	32 (59.3)	696 (62.9)	1.0 (referent)
+ perineal talc use	22 (40.7)	410 (37.1)	1.28 (0.71–2.31)
Mucinous borderline (n = 19)			
− perineal talc use	13 (68.4)	696 (62.9)	1.0 (referent)
+ perineal talc use	6 (31.6)	410 (37.1)	0.76 (0.28–2.07)

Adjusted for age, race/ethnicity, duration of oral contraceptive use and breast feeding. [1]Numbers may not add up to total cases and controls due to missing data.

TABLE IV – FREQUENCIES, MULTIVARIATE-ADJUSTED ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR PERINEAL TALC USE AND EOC RISK BY TIMING OF USE, CENTRAL VALLEY OF CALIFORNIA, 2000–2001

Timing of talc use	Cases (%) (n = 256)[1]	Controls (%) (n = 1,122)[1]	Multivariate-adjusted OR (95% CI)
Year of first use			
Never use	143 (59.1)	695 (65.6)	1.0 (referent)
Before/during 1975	52 (21.5)	206 (19.4)	1.22 (0.84–1.77)
After 1975	47 (19.4)	149 (15.0)	1.92 (1.27–2.91)
Age at first use			
Never use	143 (59.1)	695 (65.7)	1.0 (referent)
< 20 years	30 (12.4)	169 (16.0)	0.95 (0.61–1.48)
20–24 years	26 (10.7)	61 (5.8)	2.41 (1.43–4.09)
≥ 25 years	43 (17.8)	133 (12.6)	1.80 (1.19–2.73)
First use before or after first birth[2]			
Never use	113 (59.2)	631 (65.6)	1.0 (referent)
Use at or prior to first birth	36 (18.8)	229 (23.8)	0.98 (0.64–1.48)
Use after first birth	42 (22.0)	102 (10.6)	2.51 (1.63–3.87)
Years since last use			
Never use	143 (59.1)	695 (65.6)	1.0 (referent)
Current users	32 (13.2)	133 (12.5)	1.27 (0.81–1.98)
1–2 years	27 (11.2)	61 (5.8)	2.40 (1.43–4.05)
3–20 years	20 (8.3)	83 (7.8)	1.57 (0.90–2.73)
> 20 years	20 (8.3)	88 (8.3)	1.13 (0.66–1.94)

Adjusted for age, race/ethnicity, duration of oral contraceptive use and breast feeding. [1]Numbers may not add up to total cases and controls due to missing data.–[2]Parous women only.

der after 1975 compared to those reporting use prior to that date (Table IV). Higher risk was found among those reporting first use at ages after age 20 compared to those who were younger at first use (Table IV). In addition, risk was elevated among those women who used talcum powder only after the birth of their first child, while no effect was seen among those whose first use occurred before their first child was born.

In an attempt to assess latency issues, we evaluated risk of EOC by categorizing participants by the numbers of years since last reported use of talcum powder (Table IV). The highest and significant risks were found among women who had stopped using talcum powder relatively recently (1–2 years prior to interview), while those who reported last using powders in the more distant past did not experience altered risk.

An evaluation of effect modification of the talcum powder-EOC relationship by gynecologic surgery, reproductive history, exogenous hormone use and BMI is presented in Table V. Women without a tubal ligation experienced higher talcum powder-associated risks than women with a tubal ligation and this result was statistically significant (OR = 1.54; 95% CI = 1.10–2.16). The interaction coefficient for the relationship between talc use and tubal ligation was not statistically significant. There was no mod-

TABLE V – FREQUENCIES, MULTIVARIATE-ADJUSTED ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR TALC USE AND RISK OF EOC BY LEVELS OF MODIFIERS IN THE CENTRAL VALLEY OF CALIFORNIA, 2000–2001

	Cases (n = 256)[1]	Controls (n = 1,122)[1]	Multivariate-adjusted OR (95% CI)
Tubal ligation			
Never talc use	29 (56.9)	161 (54.9)	1.0 (referent)
Ever talc use	22 (43.1)	132 (45.1)	0.88 (0.46–1.68)
No tubal ligation			
Never talc use	113 (57.4)	531 (65.8)	1.0 (referent)
Ever talc use	84 (42.6)	276 (34.2)	1.54 (1.10–2.16)
Hysterectomy[2]			
Never talc use	27 (50.0)	117 (58.8)	1.0 (referent)
Ever talc use	27 (50.0)	82 (41.2)	1.79 (0.91–3.52)
No hysterectomy[3]			
Never talc use	116 (59.5)	576 (63.7)	1.0 (referent)
Ever talc use	79 (40.5)	328 (36.3)	1.33 (0.95–1.87)
Ever pregnant			
Never talc use	118 (55.9)	648 (63.0)	1.0 (referent)
Ever talc use	93 (44.1)	381 (37.0)	1.44 (1.05–1.97)
Never pregnant			
Never talc use	25 (65.8)	47 (62.7)	1.0 (referent)
Ever talc use	13 (34.2)	28 (37.3)	0.93 (0.37–2.34)
Ever parous[4]			
Never talc use	113 (57.4)	633 (62.7)	1.0 (referent)
Ever talc use	84 (42.6)	376 (37.3)	1.34 (0.97–1.85)
Nulliparous[4]			
Never talc use	5 (35.7)	15 (75.0)	1.0 (referent)
Ever talc use	9 (64.3)	5 (25.0)	4.91 (0.68–35.25)
Ever OC use			
Never talc use	72 (51.4)	422 (57.7)	1.0 (referent)
Ever talc use	68 (48.6)	309 (42.3)	1.26 (0.86–1.83)
Never OC use			
Never talc use	71 (65.1)	272 (72.9)	1.0 (referent)
Ever talc use	38 (34.9)	101 (27.1)	1.63 (1.0–2.64)
HRT[5]			
Never talc use	54 (52.4)	220 (59.9)	1.0 (referent)
Ever talc use	49 (47.6)	147 (40.1)	1.41 (0.89–2.24)
No HRT[6]			
Never talc use	89 (62.2)	472 (64.4)	1.0 (referent)
Ever talc use	54 (37.8)	261 (35.6)	1.30 (0.87–1.93)
BMI $<$ 25			
Never talc use	55 (63.2)	311 (66.5)	1.0 (referent)
Ever talc use	32 (36.8)	157 (33.5)	1.23 (0.74–2.04)
BMI $\geq$ 25			
Never talc use	85 (53.5)	358 (59.1)	1.0 (referent)
Ever talc use	74 (46.5)	248 (40.9)	1.36 (0.92–1.99)

Adjusted for age, race/ethnicity, duration of oral contraceptive use and breast feeding. [1]Numbers may not add up to total cases and controls due to missing data.–[2]Includes women with $\geq$ 2 years since hysterectomy.–[3]Includes women with $<$ 2 years since hysterectomy.–[4]Gravida women only.–[5]Includes women with one or more years of use.–[6]Includes women with never use or $<$ 1 year of use.

ification within categories of prior hysterectomy, however. Higher risks were observed among those who were ever pregnant compared to those who were never pregnant. Talcum powder-associated risk was not different within the parous and nulliparous. Talcum powder-associated risk was higher (and significant) in women who never used oral contraceptives; however, the interaction coefficient was not statistically significant. Neither BMI or hormone replacement therapy (HRT) use appeared to modify the relationship of talc use and EOC risk.

DISCUSSION

The prevalence of talc use among controls in our study (37.1%) is similar to the average percentage of use among the control populations in a review of 14 studies (36.8% calculated from data presented in original study).[21] In the current analysis as in others,[11,21–24] a larger percentage of cases *versus* controls reported perineal exposure to talc. We found a slight trend of decreasing use with increasing age in control women but our findings were not as strong as those noted by Rosenblatt *et al*.[25] Other studies[21,24] have found increased use in both cases and controls over 50 years of age compared to their counterparts less than or equal to 50 years of age. In the present study, cases less than 50 years of age were more likely to have used talc *versus* women 50 years or older (47.7% and 39.9%, respectively). Different findings in talc use patterns between the present study and previous studies may be explained by differences in study locations, study time periods and age categories. Frequency of use in the current study was similar for both the younger and older groups in controls (38.2% and 36.4%, respectively).

Talc use was higher in white non-Hispanics compared to Hispanics in this study. However, the pattern of increased use in EOC cases for both groups contributed to the overall increased risk of EOC among talc users. Differential talc use by various ethnic groups and its relation to EOC risk has not, to our knowledge, been evaluated previously.

As in other studies,[21,25] we found that talc use increased with education level, although one earlier study reported the opposite finding.[24] Other studies have compared talc use in ever married to never married women and found either similar use in both groups for cases and controls[21] or increased frequency of use in ever married women.[24] In the current analysis, talc exposure was 43.8% for ever married cases *versus* 34.4% for never married cases. However, fre-

quency of use was similar between ever married and never married controls (37.2% and 36.0%, respectively). There was much greater use of talc among those born in the United States *versus* those born outside it.

The odds ratio comparing ever use to never use in this study (OR = 1.37; CI = 1.02–1.85) is similar to the results of a recent metaanalysis that pooled 16 studies (summary RR = 1.33; CI = 1.16–1.45).[4] When stratified by hospital- *versus* population-based studies, the population studies had a summary relative risk of 1.38 (1.25–1.52).

Cornstarch use and ovarian cancer has been evaluated in a small number of case-control studies[11,21,22,26] and have been reviewed with the conclusion that no relationship exists between cornstarch and EOC, although the number of study participants using cornstarch *versus* talc was small.[13] Our study was not able to differentiate between use of perineal powders containing talc and those containing cornstarch, which may have driven the odds ratio toward the null. Type of application, including direct application on the perineum, or indirect exposure from dusting sanitary napkins, underwear and diaphragms (storage) was also not assessed.

As in other studies,[4] the present study did not find a clear dose response based on duration of use or cumulative use. Limiting the analysis of dose response to women who reported ever use of talc did not affect the results (data not shown). The lack of dose response between talc use and EOC may be explained by the inability to quantify the actual amount of talc used per application and timing of the application.[21] Cramer *et al.*[21] propose that application during ovulation may pose more risk due to the possibility of talc entrapment in inclusion cysts. Harlow *et al.*[24] found little change in odds ratios after excluding use after tubal ligation or hysterectomy in their estimate of total lifetime perineal talc applications. However, when they excluded nonovulatory periods of exposure in their calculation, there was significant increase in risk. We were unable to exclude nonovulatory periods and talcum powder use after gynecologic surgery in our cumulative use calculations.

Our analysis found that talc use and EOC risk varied by histologic subtype, as have others who found that exposure to talc is a significant risk for invasive tumors[22] and specifically for serous invasive tumors.[21] Cook *et al.*[11] also found an increased risk of serous tumors (including both invasive and borderline) in talc users *versus* nonusers. Gertig *et al.*[27] have suggested that there are pathologic similarities between serous adenocarcinomas and mesothelioma that may explain findings of increase risk for serous invasive tumors in talc users. Harlow *et al.*[24] reported a significant increase in risk of either endometrioid or borderline tumors with talc use and suggest that variation in risk among histologic subtypes may be due to chance or a foreign-body effect unique to specific subtypes.

In a study of the mineral and chemical characterization of consumer talcum powder formulated prior to June 1973, almost half of the samples tested contained 1 of the 3 asbestos group minerals.[1] In 1976, talcum powder manufacturers instituted voluntary guidelines to prevent asbestos contamination in talc products,[24] but we did not find an increase in EOC risk with talc use on or before 1975; rather, we found that risk of EOC increased with use after 1975, which may be related to the recency of use. Harlow *et al.*[24] observed ovarian cancer risk was increased in women using talc products before 1960, although Chang and Risch[22] found no relationship between risk and use either before or after 1970.

In the current analysis, a statistically significant increase in EOC risk occurred with first use after age 20 compared to first use at younger ages. Controlling for recency of use did not change this finding. Other studies have reported either no trend with age at first use[21] or increased risk of EOC with first use at younger than age 20 and older than age 25.[24] Disagreement in findings between studies may be due to differences in age distributions and talc use patterns among study participants. Although we cannot directly assess risk during ovulatory *versus* nonovulatory periods, our findings of increased risk in adult women support the hypotheses of increased EOC risk with talc exposure during ovulatory periods and in parous reproductive tracts.

Cramer *et al.*[21] found that EOC risk was increased in parous women with talc use occurring before first birth, suggesting that prepregnancy ovarian tissue may be more vulnerable to talc damage because it has not undergone stromal differentiation (decidual reaction that occurs during pregnancy). However, their reference group was all parous women. In this analysis, we stratified parous women by never use, use before first birth and use after first birth. We found increased risk after first pregnancy. Anatomical changes in the genital tract after pregnancy may increase the possibility of talc migration to the ovaries.[28] Harlow *et al.*[24] suggest that pregnancy may increase risk due to its effect of increasing the size of the cervical opening into the uterus. In the current study, perineal talc use had no apparent impact on EOC risk among those women who had never been pregnant and parity was difficult to evaluate because of the small number of nulliparous women. In 2 prospective studies of talc use and EOC risk, there was no significant difference in parity between users and nonusers of talc.[25,27]

Harlow *et al.*[24] reported a significant increase in EOC risk if perineal talc use occurred in the last 6 months. We also found that recent users were at increased risk (even when we controlled for duration of use). It is noteworthy that a significant latency effect is well documented for asbestos exposure and development of both pleural and peritoneal mesotheliomas[29] while there appears to be no latency with talcum powder. The asbestos association has been reported from an occupational cohort mortality study where exposure is indirect and not the result of direct application, as is the case for talcum powder.[30] This may explain the differences between observed patterns of latency for asbestos and talcum powder. Additionally, risk of EOC with talc use may not be due to talc's chemical similarity to asbestos but rather due to the ovary's unique function, resulting in vulnerability to carcinogenesis from particulates such as talc.[8] In a study of gynecologic surgery and EOC, Green *et al.*[31] found that women reporting fallopian tubal occlusion, through tubal ligation or hysterectomy, were at decreased risk for developing EOC. They concluded that surgical tubal occlusion decreased EOC risk by preventing contaminants from reaching the ovary. Ness and Cottreau[32] proposed that the inflammatory response of the ovarian epithelium to various irritants may result in ovarian mutagenesis, tumor growth and tumor invasiveness. Cramer *et al.*[21] reported no association between EOC risk and talc use in women with a tubal ligation; however, risk remained nonsignificantly elevated in women with a hysterectomy. A recent prospective study[27] found that EOC risk in talc users was not modified by either tubal ligation or hysterectomy. The analysis was not able to determine the timing of talc use (before or after surgery). In a hospital-based case-control study, Wong *et al.*[33] found that risk of EOC with talc use was increased in women without gynecologic surgery and decreased in women with a history of tubal ligation or hysterectomy but neither finding was significant. They also were unable to delineate use before or after gynecologic surgery. Tubal ligation may limit a woman's exposure to contaminants more than hysterectomy since it is usually performed earlier in a woman's reproductive history, while she is still ovulating.[34]

Oral contraceptives (OCs) act by suppression of ovulation and the fact that elevated risks were found in those talcum powder users that never used OCs in this study suggests that uninterrupted ovulation with associated formation of inclusion cysts may enhance the impact that talcum powder may have on ovarian carcinogenesis. Unlike our study, however, Cramer *et al.*[21] and Harlow *et al.*[24] reported that OCs had no effect on talc use and EOC risk. A prospective study of talc use and ovarian cancer also found that the prevalence of OC use was similar in both users and nonusers of talc. However, these studies also reported lower percentages of OC use among both cases and controls (talc users and nonusers) than was found in the present study.

Our analysis found that BMI did not modify the risk associated with talc use and EOC in agreement with Cramer *et al.*[21] Talc use

was greater in women with a high *versus* low BMI for both cases and controls but the difference was not significant. Rosenblatt *et al*.[25] in a prospective study found that women in the highest BMI quartile were more likely to use perineal talc. They concluded that since some studies have found an increased risk for ovarian cancer in obese women, BMI may be a confounder of talc use and ovarian cancer risk. Harlow *et al*.,[24] however, reported no differential use of talc between leaner and heavier controls.

There are several limitations to this study that may limit interpretation of the findings. The sample size was relatively small and the response fraction lower than ideal. However, we have observed the same or similar relationships in our study between several risk factors such as OC use and parity, as has been observed in several earlier studies. Recall bias has also been implicated as a limitation in studies of talc and ovarian cancer.[35] However, findings in a prospective study, the Nurses' Health Study, in which exposure data were collected prior to diagnosis and hence free of recall bias, were similar to the present study finding for talc use and serous invasive ovarian cancer.[27] It has also been suggested that use of talc is habitual *versus* memorable and not likely to be subject to recall bias.[35] Huncharek *et al*.[4] suggested that the positive relationship between talc use and EOC risk found in a review of epidemiologic studies may also be explained by a treatment effect in prevalent cases. The present study used incident cases exclusively. The present analysis was also limited due to our inability to exclude use during nonovulatory periods and posttubal ligation or hysterectomy, nor were we able to differentiate between various formulations.

Research has provided little biologic or experimental evidence to support a relationship between talcum powder use and ovarian cancer risk. However, given the suggestive though uncertain role of talcum powder and EOC found in epidemiologic studies, including the present study, users should exercise prudence in reducing or eliminating use. In this instance, the precautionary principle should be invoked, especially given that this is a serious form of cancer, usually associated with a poor prognosis, with no current effective screening tool, steady incidence rates during the last quarter century and no prospect for successful therapy. Unlike other forms of environmental exposures, talcum powder use is easily avoidable.

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Exhibit 41

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Hormonal factors and the risk of invasive ovarian cancer: a population-based case-control study

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Objective: To examine the influence of hormone-related factors on the risk of invasive epithelial ovarian cancer (ovarian cancer).

Design: Population-based case–control study using in-person interviews.

Setting: Academic department of preventive medicine.

Patient(s): Four hundred seventy-seven ovarian cancer patients and 660 controls.

Intervention(s): None.

Main Outcome Measure(s): Numbers of and ages at births, oral contraceptive use, and use of menopausal hormone therapy.

Result(s): Compared with nulliparous women, women whose only (last) birth was after age 35 years had an estimated 51% (95% confidence interval: 21%–70%) reduction in risk. If this birth occurred earlier, the reduction in risk was progressively less. Additional (earlier) births reduced the risk further. Oral contraceptive use also reduced risk. Increased body mass index increased risk, but this effect was confined to localized disease and is likely to be a diagnostic bias, as a consequence of other problems associated with being overweight and in itself having no etiological significance.

Conclusion(s): If the major protective effect of a late birth can be confirmed, our most challenging task will be to understand the mechanism to develop a chemoprevention approach to exploit this finding. (Fertil Steril® 2004;82:186–95. ©2004 by American Society for Reproductive Medicine.)

Key Words: Ovarian cancer, parity, estrogen therapy, oral contraceptives

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Epidemiological studies have consistently found that the risk of invasive epithelial ovarian cancer (ovarian cancer) is significantly decreased with increasing numbers of births, and Fathalla (1) proposed that the mechanism of this effect was the interruption of the tearing of the ovarian surface epithelium (OSE) with each ovulation (the *incessant-ovulation hypothesis*). The OSE is regarded by most, but not all, pathologists as the tissue of origin of ovarian cancer (2), and it was the increased cell division of the OSE that is involved in the repair of the surface after each ovulation that was presumed to be a major factor increasing ovarian cancer risk. The initial epidemiological findings of a significant protective effect of oral contraceptive (OC) use (3–5) appeared to provide further support for the incessant-ovulation hypothesis.

Since that time, a number of epidemiological and experimental findings have demonstrated that the incessant-ovulation hypothesis does not provide a comprehensive explanation of the etiology of ovarian cancer. These findings include a greater reduction in risk with first birth than with subsequent births (6), a greater reduction in risk with any birth than with a year of OC use (6), a continued rise in incidence with age after menopause (7), and a much lower ovarian cancer rate in "traditional" Japanese women than would be predicted by the hypothesis. The experimental finding of a stimulatory effect of estrogen (E) on benign ovarian tumor cells but an inhibitory effect of progesterone (8) suggests a possible unifying hypothesis for the etiology of ovarian cancer (8, 9).

We report here our investigations of these issues in a large population-based case–control study conducted in Los Angeles.

Epidemiological studies of epithelial ovarian cancer frequently include low malignant potential tumors with invasive tumors without distinction. We have not done this here, because molecular genetic studies suggest that such tumors are not part of a disease continuum but represent separate disease entities, and their age incidence is radically different (10).

MATERIALS AND METHODS

The study was approved by the institutional review board of the Keck School of Medicine of the University of Southern California. Informed consent was obtained from each patient and control before her interview.

Patient and Control Selection

Eligible patients were English-speaking non-Asian female residents of Los Angeles County who had histologically confirmed ovarian cancer or borderline (low malignant potential; LMP) ovarian tumors that were first diagnosed between 18 and 74 years of age, from October 1992 through October 1998. Patients and controls with previously diagnosed cancer (except nonmelanoma skin cancer) were not eligible. The reason for excluding such women is that treatment for a previous cancer may delay the appearance of an ovarian cancer or alternatively lead to its earlier diagnosis. The latter phenomenon was clearly seen in our case identification, in which 31 ovarian tumors were diagnosed during the workup of another cancer (most commonly endometrial cancer); these cases are not included in the numbers given in the next paragraph. The cases were identified by the Cancer Surveillance Program, the cancer registry covering all residents of Los Angeles County. Patients with borderline endometrioid tumors were excluded from the study because this subtype is classified as a benign tumor by the International Agency for Research on Cancer and is not ascertained by the Cancer Surveillance Program or other certified North American cancer registries.

A total of 1,442 patients meeting the pathological case definition were identified by the Cancer Surveillance Program. We identified 139 of these patients as not English speaking, leaving 1,303 eligible patients. Of these, 291 patients had died or were too ill to be interviewed by the time that we contacted their physicians; the patients' physicians refused permission to contact an additional 65 patients; 31 patients were no longer residents of Los Angeles County and had moved too far away to be interviewed in person; 66 patients could not be located; and 173 patients declined to be interviewed. Interviews were conducted with 677 patients (52% of all patients and 80% of patients approached): 491 of these were classified as having ovarian cancer, and 186, as having LMP tumors.

Controls were English-speaking non-Asian women with at least one intact ovary, individually matched with patients on race and ethnicity (African-American, Latina, non-Latina White) and date of birth (±3 years). Initially, a neighborhood control was sought by one of our staff who physically canvassed the neighborhood using a systematic algorithm based on the address of the patient. If the first eligible match refused to participate, the second eligible match in the sequence was asked, and so on. Letters were left when no one was home, and follow-up by mail, telephone, and further visits to the neighborhood continued until either an eligible control agreed to be interviewed or 150 housing units had been screened. For patients aged >65 years, if no willing control could be found in the first 100 housing units, a control was simultaneously sought among a random sample of female residents of Los Angeles County aged >65 years who were provided to us by the Health Care Financing Administration. The Health Care Financing Administration control was matched on the patient's zip code, race and ethnicity, and date of birth (closest to that of the patient).

When the control had an ovary-sparing hysterectomy before her reference date (12 months before the diagnosis of her matching patient) but was matched with a patient who had not had a hysterectomy, a second control who had not had a hysterectomy by that date was sought. Altogether, 664 controls were successfully interviewed by the closing date of the study. The first eligible match was interviewed for 70% of the patients, and the second match, for another 21%. At the termination of the study, 530 of the interviewed patients were matched with at least 1 interviewed control, 136 of the interviewed patients had no matching interviewed control, and 104 interviewed controls were matches for patients who had not been successfully interviewed or turned out to be ineligible. In total, 30% of eligible controls identified declined to be interviewed (the same proportion as that of first eligible controls declining to be interviewed).

This report is confined to ovarian cancer patients, but use is made of all interviewed controls.

Risk Factor Assessment: Questionnaires

Each patient was interviewed in person by using a comprehensive questionnaire covering medical, gynecological, reproductive, and certain aspects of personal lifestyle history, up to 12 months before her diagnosis date (her reference date). Calendars were used to chart major life events and reproductive and contraceptive histories. Each control was interviewed in the same manner, with the pseudo-reference date taken as the reference date of her matched patient.

Data Analysis

Statistical analyses were conducted by standard statistical methods (11) including multivariate logistic regression (EPILOG statistical package program; EpiCenter Software, Pasadena, CA). Although the study was designed as a matched case–control study, a significant number of patients did not

have a matched control, and we wished to include the controls of the LMP patients, so a multivariate unconditional logistic regression analysis approach was adopted. Adjustments were made for three race and ethnicity groups (African-Americans, Latinas, non-Latina Whites), 5-year age groups, four levels of socioeconomic status (SES) according to census tract of residence at the time of diagnosis (12), and four levels of education.

All the reported risk estimates were in addition adjusted for the following factors: family history of ovarian cancer (mother or sister; yes/no), tubal ligation (yes/no), use of genital area talc (yes/no), usual body mass index (BMI) during 5 years before reference date (kilograms per meter square; categorical variable), nulliparity (yes/no), age at last (term) birth (ALB; ALB at 35+ years and per 5-year group before 35+ years; continuous), number of additional births (i.e., zero, or total births minus 1, whichever is the greater; continuous variable), number of incomplete pregnancies (continuous), duration of OC use (months; continuous), type of menopause (premenopausal vs. natural vs. surgical), age at natural menopause (5-year age groups; continuous), age at surgical menopause (5-year age groups; continuous), and duration of menopausal hormone therapy use (E–progestin therapy [EPT] use by hysterectomized women, EPT use by naturally menopausal women, E therapy [ET] use by hysterectomized women, and ET use by naturally menopausal women; all continuous). All statistical significance values (*P* values) quoted are two sided and are standard χ^2 analyses based on differences in log likelihoods (11).

Women undergoing a hysterectomy without a bilateral oophorectomy (simple hysterectomy) before menopause were classified as having a surgical menopause. For naturally menopausal women, age at menopause was estimated as follows: for a woman taking OCs, age at menopause was taken as the end of the period of OC use, if no "natural" menstruation occurred thereafter. Natural menstruation was taken to mean menstruating and not using OCs or menopausal hormone therapy (HT) at the time. For a woman taking HT to within 3 months before her reported age at last menstrual period, we set her age of menopause at the date on which she began HT use, with the rationale that HT use was started because of menopausal symptoms.

We elsewhere have given the justification for this approach to setting age at menopause (13). Essentially, age at last menstrual period cannot be used to uniformly estimate age at menopause because women who use sequential EPT usually continue to have monthly menstrual periods, irrespective of their ovarian function, and women on ET and continuous-combined EPT can rarely distinguish breakthrough bleeding from ovarian function–determined menses.

The E component of OCs was considered high if the dose of ethinyl estradiol was >35 μg or if the dose of mestranol was >70 μg. The progestin component was considered high if the dose was equivalent to ≥0.30 mg of DL-norgestrel. The conversion to assumed equivalent DL-norgestrel doses was done based on the results of studies of Greenblatt, as described in Dickey and Stone (14).

TABLE 1

Demographic characteristics of ovarian cancer patients and controls.

	Controls		Patients	
Characteristics	n	%	n	%
Race/ethnicity				
African American	50	7.6	44	9.2
Latina	92	13.9	61	12.8
White	518	78.5	372	78.0
Total	660		477	
Age (y)				
<35	75	11.4	20	4.2
35–44	98	14.8	62	13.0
45–54	203	30.8	140	29.4
55–64	138	20.9	144	30.2
65+	146	22.1	111	23.2
Socioeconomic status				
1 (high)	242	36.7	176	36.9
2	188	28.5	117	24.5
3	100	15.2	71	14.9
4 (low)	130	19.7	113	23.7
Education				
<High school	41	6.2	44	9.2
High school	174	26.4	154	32.3
Some further training	340	51.5	229	48.0
College graduate	105	15.9	50	10.5

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RESULTS

Eleven patients were found to have had a previous cancer at interview and were thus deemed ineligible. Three ovarian cancer patients and two controls were eliminated because of reported age of <35 years at natural menopause, and two additional controls were excluded because of contradictory data. The race and ethnicity, age, SES, and education of the remaining patients and controls are shown in Table 1. The patients are older than the controls; this is because LMP tumors occur at a younger age than do invasive tumors, and the controls were matched to both LMP tumor patients and ovarian cancer patients.

Analysis of family history (in mother or sister) of ovarian cancer, tubal ligation, and use of genital area talc showed the well-known effects: odds ratios (ORs) were 3.78 for family history, 0.82 for tubal ligation, and 1.60 for talc (Table 2). Although the reduced risk associated with tubal ligation was not statistically significant in these data, all three factors have been included in our statistical model because they are well-established risk factors. Table 2 also shows that risk was increased in women with BMI (weight in kg/height in

TABLE 2

Analysis of some known risk factors for ovarian cancer.

Risk factor	Controls	Patients	Adjusted OR[a]	95% CI	Statistical significance χ^2 (df)	Statistical significance *P* value
Family history of ovarian cancer						
No	648	448	1.00			
Yes	12	29	3.78	1.83–7.80	14.33 (1)	.0002
Tubal ligation						
No	579	431	1.00			
Yes	81	46	0.82	0.53–1.26	0.86 (1)	.35
Genital area talc						
No	544	349	1.00			
Yes	116	128	1.60	1.18–2.18	9.05 (1)	.0026
BMI (kg/m^2)[b]						
<25	397	261	1.00			
25–29	165	120	0.97	0.71–1.33		
30–34	60	56	1.29	0.83–1.99		
35+	38	40	1.46	0.87–2.44	3.44 (3)	.32

Note: CI = confidence interval; df = degrees of freedom; ALB = age at last (term) birth; EPTH = EPT used by hysterectomized women; EPTM = EPT used by naturally menopausal women; ETH = ET used by hysterectomized women; ETM = ET used by naturally menopausal women; SES = socioeconomic status.

[a] Adjusted for ethnicity (3 groups), age (9), SES (4), education (4), family history of ovarian cancer (2), tubal ligation (2), use of genital area talc (2), BMI (4), nulliparity (2), ALB (at 35+ y, per 5-y group before 35+ y), number of additional births (continuous), number of incomplete pregnancies (continuous), OC use (continuous), menopausal status (3), age at natural menopause (5-y age groups, continuous), age at surgical menopause (5-y age groups, continuous), ETM (continuous), ETH (continuous), EPTM (continuous), and EPTH (continuous).

[b] Calculated with usual weight during 5 y before reference date.

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meters squared) of ≥30 kg/m^2; although this result was also not statistically significant, it too has been included in our final statistical model because there is substantial evidence for this effect (15).

Women who had four or more (term) births had a 64% reduced risk of ovarian cancer compared with nulliparous women (Table 3). Risk declined with increasing numbers of births (OR = 0.83 per birth; $\chi^2_1 = 17.59$; $P<.0001$). The effect of the first birth was greater than that of succeeding births (OR = 0.68 compared with OR = 0.86), although this difference was not statistically significant.

The OR associated with ALB are also shown in Table 3. There is good evidence that the later the ALB, the lower the risk. Fitting nulliparity (nulliparous vs. parous) and ALB (among parous women) gave $\chi^2_4 = 21.15$ ($P=.0003$); fitting nulliparity and ALB as a continuous variable gave $\chi^2_2 = 19.93$ ($P<.0001$). Each additional birth reduced risk by 10% ($\chi^2_1 = 3.10$; $P=.078$). We observed a strong positive relationship between late age at first birth and late ALB and were not able to clearly distinguish between them. This was so even when we restricted analysis to women with two or more children. We kept ALB in the analysis because the result was clearer (steady effect in most subgroups we studied).

As we have seen, the effect of fitting additional births after fitting ALB was not statistically significant, but increasing numbers of incomplete pregnancies was associated with a statistically significant reduced risk of ovarian cancer ($\chi^2_1 = 4.29$; $P=.038$; Table 3), and both variables (additional births and incomplete pregnancies) have been retained in the statistical model. There was no effect of age at incomplete pregnancy, and, in particular, replacing ALB with age at last pregnancy led to a reduction in the fit of the model to the data. Fitting separate terms for spontaneous and induced abortions showed a stronger protective effect of reported induced abortions, but the difference was not statistically significant.

Breast-feeding the last baby was associated with a 23% reduction in risk, but the result was not statistically significant, and breast-feeding other babies was not associated with any reduction risk. We did not include breast-feeding in our final statistical model.

Oral contraceptive use was associated with a statistically significant protective effect (Table 4). There was a 5.8% protective effect per year of OC use ($\chi^2_1 = 16.72$; $P<.0001$). There was no differential effect of age at OC use; dividing the age at use into four categories (<25 years, 25–29 years, 30–34 years, and 35+ years) was associated with no pattern of effect (difference from single term, $\chi^2_3 = 5.54$; $P=.14$). Categorizing OCs into four groups based on their E and progestin content showed no significant differences ($\chi^2_3 =$

TABLE 3

Complete and incomplete pregnancies and risk of ovarian cancer.

Variable	Controls	Patients	Adjusted OR[a]	95% CI	Statistical significance χ^2 (df)	Statistical significance *P* value
Births						
0	139	120	1.00			
1	103	65	0.62	0.40–0.96[b]		
2	174	127	0.62	0.42–0.90[b]		
3	124	90	0.55	0.36–0.84[b]		
4+	120	75	0.36	0.22–0.57[b]	19.46 (4)	.0006
Per birth			0.83	0.76–0.91[b]	17.59 (1)	<.0001
First birth			0.68	0.48–0.97[c]		
Per additional birth			0.86	0.78–0.96[c]	18.93 (2)	<.0001
ALB (y)						
Nulliparous	139	120	1.00			
<25	92	94	0.84	0.55–1.29[d]		
25–29	170	110	0.55	0.37–0.81[d]		
30–24	158	100	0.50	0.33–0.74[d]		
35+	101	53	0.42	0.26–0.67[d]	21.15 (4)	.0003
ALB at 35+			0.49	0.30–0.79		
Per 5-y group before age 35 y			1.18	1.01–1.38	19.93 (2)	<.0001
Per additional birth			0.90	0.80–1.01	3.10 (1)	.078
Incomplete pregnancies						
0	402	300	1.00			
1	154	98	0.86	0.62–1.17		
2	58	40	0.93	0.59–1.48		
3	23	12	0.77	0.36–1.66		
4+	22	7	0.36	0.14–0.92	5.91 (4)	.21
Per incomplete pregnancy			0.88	0.77–0.99	4.29 (1)	.038

Note: CI = confidence interval; df = degrees of freedom; ALB = age at last (term) birth; EPTH = EPT used by hysterectomized women; EPTM = EPT used by naturally menopausal women; ETH = ET used by hysterectomized women; ETM = ET used by naturally menopausal women; SES = socioeconomic status.

[a] Adjusted for ethnicity (3 groups), age (9), SES (4), education (4), family history of ovarian cancer (2), tubal ligation (2), use of genital area talc (2), BMI (4), nulliparity (2), ALB (at 35+ y, per 5-y group before 35+ y), number of additional births (continuous), number of incomplete pregnancies (continuous), OC use (continuous), menopausal status (3), age at natural menopause (5-y age groups, continuous), age at surgical menopause (5-y age groups, continuous), ETM (continuous), ETH (continuous), EPTM (continuous), and EPTH (continuous).

[b] Adjustment variables excluded: nulliparity, ALB, and additional births.

[c] Adjustment variables excluded: nulliparity and ALB.

[d] Adjustment variables excluded: nulliparity and additional births.

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4.90; *P*=.18), although the reduction in risk was greater with high-progestin OCs and was most marked in the low E with high-progestin OCs (Table 4).

No differences were observed between patients and controls in the occurrence of difficulties in getting pregnant or in the use of fertility drugs (data not shown).

No effect of age at menarche was seen (data not shown).

There was a trend for later age at natural menopause to be associated with increased risk (OR = 1.19 per 5 years later; Table 5), and there was also a trend for later age at hysterectomy to be associated with an increased risk of ovarian cancer (OR = 1.10 per 5 years later; Table 5), but the results were not statistically significant.

Menopausal ET use in naturally postmenopausal women was associated with an increase in risk of ovarian cancer (OR = 1.16 per 5 years of use), but the result was not statistically significant (Table 5). Estrogen-progestin therapy use was not associated with any increase in risk. The results for ET use in hysterectomized women were very similar (OR = 1.11 per 5 years of use) to those seen with naturally menopausal women (Table 5). The numbers of EPT users in hysterectomized women were too small for meaningful analysis.

Stage at diagnosis was recorded by the cancer registry for 464 (97.3%) of the 477 patients: 100 (21.6%) of the patients were classified by the Surveillance, Epidemiology, and End Results registry as localized (confined entirely to the organ of origin), and 364 (78.4%), as having regional or distant

TABLE 4

Oral contraceptive (OC) use and risk of ovarian cancer.

Variable	Controls	Patients	Adjusted OR[a]	95% CI	Statistical significance χ^2 (df)	Statistical significance *P* value
OC use (y)						
Never	245	222	1.00			
<5	246	169	1.00	0.72–1.39		
5–9	90	51	0.72	0.46–1.13		
10+	79	35	0.48	0.29–0.78		
Per y of use			0.94	0.91–0.97	16.72 (1)	<.0001
OC formulation						
High E + high P	40	20	0.88	0.81–0.97		
High E + low P	70	41	0.94	0.88–1.00		
Low E + high P	10	4	0.66	0.36–1.21		
Low E + low P	203	132	0.95	0.92–0.99		
Unknown	151	100	0.96	0.90–1.02	21.95 (5)	.0005

Note: CI = confidence interval; df = degrees of freedom; ALB = age at last (term) birth; EPTH = EPT used by hysterectomized women; EPTM = EPT used by naturally menopausal women; ETH = ET used by hysterectomized women; ETM = ET used by naturally menopausal women; SES = socioeconomic status.

[a] Adjusted for ethnicity (3 groups), age (9), SES (4), education (4), family history of ovarian cancer (2), tubal ligation (2), use of genital area talc (2), BMI (4), nulliparity (2), ALB (at 35+ y, per 5-y group before 35+ y), number of additional births (continuous), number of incomplete pregnancies (continuous), OC use (continuous), menopausal status (3), age at natural menopause (5-y age groups, continuous), age at surgical menopause (5-y age groups, continuous), ETM (continuous), ETH (continuous), EPTM (continuous), and EPTH (continuous).

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(more extensive) disease. Analyzing the two groups of patients separately showed important differences in the estimates of the effects of BMI. The odds ratios increased sharply over the four increasing BMI categories (<25, 25–29, 30–34, and 35+) for localized disease, from 1.00 to 1.42 to 2.66 and finally to 3.43 for BMI of 35+ (χ^2_3 = 11.64; *P*=.0087). There was only a very weak, not statistically significant relationship between BMI and risk of regional or distant disease; the odds ratios over the same increasing BMI categories were 1.00, 0.89, 1.07, and 1.16. The effect of ET was also more evident for localized disease: for localized disease, the ORs for 5 years of use were 1.51 and 1.22 for natural menopause and hysterectomized women, respectively, whereas for regional or distant disease, the ORs were 1.11 and 1.10, respectively.

The patients were histologically classified as follows: 324 as serous, 92 as endometrioid (including clear cell), 42 as mucinous and 19 as other (or unknown). There was a marked relationship between stage and histology: for serous tumors, 12% were localized; for endometrioid, 42%; and for mucinous, 55%. With endometrioid tumors, a last birth at age 35+ years reduced risk by 80%, whereas for serous tumors, the reduction in risk was only 36% (*P* value for difference in effect, .039). There were too few data to make further meaningful comparisons.

DISCUSSION

The most striking finding in this study was a possible major effect of ALB; the later the ALB, the lower the risk of ovarian cancer. Compared with a nulliparous woman, the overall risk of ovarian cancer in a woman with a last birth after age 35 years was reduced by 58%, with a reduction of 51% due solely to the last birth. A last birth before age 25 years was associated with only a 16% reduced risk of ovarian cancer; even after adjusting for numbers of births, this only increased to 20%. Additional births (before the last) reduced risk further by some 10% per birth.

These results for ALB must, however, be regarded as somewhat tentative for the following reason: fitting numbers of births as a single linear term was significantly associated with decreasing ovarian cancer risk (Table 3; χ^2_1 = 17.59). Including ALB with numbers of births improved this fit (χ^2_3 = 23.03), but this improvement is not quite statistically significant (χ^2_2 = 5.44, *P*=.066). If, based on the results of this and previous studies, we regard the effect of first birth as being greater than that of subsequent births, the fit to the data is measured by χ^2_2 = 18.93 (Table 3). In comparison to this, fitting ALB in addition to number of births is just statistically significant (χ^2_1 = 4.10, *P*=.043).

Age at last pregnancy provided a less good fit to our data than ALB.

Oral contraceptive use reduced ovarian cancer risk by approximately 6% per year of use, a smaller effect than seen with births. Age at last use of OCs and whether the use occurred before or after last pregnancy had no differential effect on risk. There was no evidence of an increased effect of OC use with increasing age at use. There was a greater protective effect with OCs containing a high-dose progestin, especially if the high-dose progestin was combined with a low dose of ethinyl estradiol, but these results were not statistically significant.

Although the differences were not statistically significant, both additional births and OC use were associated with a greater protective effect against ovarian cancers diagnosed at younger ages; reductions in risk in cancer diagnosed at age <55 years, at 55–64 years, and at 65+ years were per additional birth and per year of OC use (respectively): 0.79 and 0.92, 0.82 and 0.96, and 1.05 and 0.98. This effect was not seen with ALB.

Earlier ages at both natural and surgical menopause were associated with decreased risks of ovarian cancer. Postmenopausal ET was found to increase ovarian cancer risk by approximately 11%–16% per 5 years of use in naturally and surgically menopausal women. The effect was smaller in regional or distant disease. Estrogen-progestin therapy use was effectively confined to naturally postmenopausal

TABLE 5

Menopause and use of menopausal ET and EPT and risk of ovarian cancer.

Variable	Controls	Patients	Adjusted OR[a]	95% CI	Statistical significance χ^2 (df)	Statistical significance *P* value
Natural menopause						
Age at natural menopause (y)						
<45	44	31	1.00			
45–49	94	74	1.29	0.71–2.35		
50–54	129	113	1.67	0.94–2.99		
≥55	32	23	1.44	0.65–3.17		
Per 5 y			1.19	0.95–1.49	2.29 (1)	.13
ET (mo)						
0–12	263	215	1.00			
13–60	19	11	0.71	0.32–1.61		
61+	17	15	1.36	0.62–2.96		
Per 5 y			1.16	0.92–1.48	1.53 (1)	.22
EPT (mo)						
0–12	188	175	1.00			
13–60	54	26	0.60	0.34–1.05		
61+	57	40	0.90	0.55–1.48		
Per 5 y			0.97	0.77–1.23	0.05 (1)	.82
Surgical menopause						
Age at hysterectomy (y)						
<35	21	25	1.00			
35–39	18	23	1.30	0.52–3.22		
40–44	23	31	1.23	0.52–2.91		
≥45	8	12	1.38	0.44–4.32		
Per 5 y			1.10	0.79–1.53	0.30 (1)	.59
ET (mo)						
0–12	36	40	1.00			
13–60	12	17	1.12	0.44–2.86		
61+	22	34	1.56	0.72–3.41		
Per 5 y			1.11	0.92–1.35	1.19 (1)	.28
EPT (mo)						
0–12	67	84	1.00			
13–60	1	4	5.04	0.50–51.22		
61+	2	3	1.13	0.15–8.31		
Per 5 y			1.30	0.63–2.67	0.60 (1)	.44

Note: CI = confidence interval; df = degrees of freedom; EPT = E-progestin therapy; SES = socioeconomic status; ALB = age at last (term) birth; EPTH = EPT used by hysterectomized women; EPTM = EPT used by naturally menopausal women; ETH = ET used by hysterectomized women; ETM = ET used by naturally menopausal women

[a] Adjusted for ethnicity (3 groups), age (9), SES (4), education (4), family history of ovarian cancer (2), tubal ligation (2), use of genital area talc (2), BMI (4), nulliparity (2), ALB (at 35+ y, per 5-y group before 35+ y), number of additional births (continuous), number of incomplete pregnancies (continuous), OC use (continuous), menopausal status (3), age at natural menopause (5-y age groups, continuous), age at surgical menopause (5-y age groups, continuous), ETM (continuous), ETH (continuous), EPTM (continuous), and EPTH (continuous).

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women, and in these women, no effect of EPT use was evident.

An increased BMI was significantly associated with increased risk of localized ovarian cancer, but there was only a slight association with regional or distant disease. This suggests to us that it is likely that increasing BMI may bring a woman more frequently to medical attention and that localized disease is then found incidentally during the workup of other problems (such as irregular bleeding). At present, we do not have adequate data to investigate this further.

The above results were generally seen with the different histologic varieties of ovarian cancer. The difference that was suggestive of a real effect was the observation that endometrioid (and clear cell) tumors were more profoundly reduced with ALB at age 35+ years (80% reduction, compared with 36% for serous tumors); this needs confirmation.

We investigated to what extent some of these results could be due to the fact that we only managed to interview 52% of the potential patients (80% of those approached). There were differences between the interviewed and noninterviewed patients on a number of factors: those interviewed were younger (age <45 years: 17.2% vs. 14.5%; 45–54 years: 29.4% vs. 19.2%; 55+ years: 53.4% vs. 66.3%), were of higher SES (SES 1: 36.9% vs. 20.1%; SES 2–3: 39.4% vs. 47.1%; SES 4: 23.7% vs. 32.8%), and had a different ethnic mix (African-American: 9.2% vs. 17.7%; Latina: 12.8% vs. 8.3%; White: 78.0% vs. 74.0%). These differences should not matter because all analyses adjusted for age, SES, and ethnicity. However, the interviewed patients were biased toward "earlier" stage at diagnosis (localized: 22% vs. 14%), and as we have seen, this does affect the overall relationship between BMI and ovarian cancer risk because it is only the localized patients that show any such relationship. As we discussed above, this observed effect of BMI requires further investigation.

How do these results fit in with previous studies? A profound effect of ALB in close agreement with our results was seen in the recently published study of Whiteman et al. (16). An effect of age at births was first noted in the four US population-based case–control studies analyzed by Whittemore et al. (6); those investigators observed a reduced risk of ovarian cancer with late age at first birth. As noted above, in the study reported here, we observed a strong positive relationship between late age at first birth and late ALB, and the result found by Whittemore et al. (6) is thus likely to be supportive of an effect of late ALB. Titus-Ernstoff et al. (17) also observed a trend of increased protective effect with late ALB.

No effect of ALB was seen in a Canadian population-based case–control study (18) or in a Swedish population-based case–control study (19). We have no explanation for these apparently contradictory results. It may be that the high proportion (40%) of immigrants in the Canadian study, which was not adjusted for in the analysis, produced their result. The Swedish study used a mailed questionnaire: were the inherent limitations in such an approach a contributing factor to their results? No other published studies had covariate data available (in particular, OC data) to adjust their estimates of the effects of age at births, and thus this variable is not interpretable in other studies.

Oral contraceptive use has been consistently found to be associated with a reduced risk of ovarian cancer (3–6, 9, 18, 20, 21). The results from cohort and population-based case–control studies suggest a reduction in risk of around 8%, with a slightly reduced risk with longer time since last use. A recent reanalysis of the Cancer and Steroid Hormone Study (9) suggested that OC formulations with a "high-dose/potency" progestin may be associated with a greater reduction in risk than those with a "low-dose/potency" progestin. We observed this effect, but our results were not statistically significant, and Ness et al. (20) did not observe such an effect. Further data are needed to adequately evaluate this progestin dose issue. No current commonly prescribed OCs contain a high-dose progestin.

Hysterectomy consistently has been found to be a significant protective factor against ovarian cancer (see, for example, Irwin et al. [22], Hankinson et al. [23], and Green et al. [24]). We did not fully address this issue in our study because only women who were sure that they had not had a bilateral oophorectomy at the time of their hysterectomy were included as controls. This meant that we will have excluded some women as controls who did not have a bilateral oophorectomy at the time of their hysterectomy but who were not sure that this was the case: this leads to a reduction in the estimate of any reduction in risk associated with hysterectomy, and we considered hysterectomy to be a variable that needed to be adjusted for in all our analyses, not one for which we could obtain a valid estimate of protective effect. It was not practical for us to avoid this problem by obtaining operation records for all hysterectomized potential controls.

We did, however, see a much greater protective effect from an earlier rather than a later hysterectomy, and this strongly suggests a real age-dependent protective effect of hysterectomy. This age-dependent protective effect of hysterectomy has been consistently found and, in particular, was found in the Nurses Health Study prospective cohort (23), in which recall bias is not an issue: in this study, a 52% reduction in risk was found with hysterectomy before age 45 years, compared with a 24% reduction with hysterectomy after this age.

The age incidence of ovarian cancer shows a marked slowing of the rate of increase around age 50 years (even after allowing for the effect of oophorectomies, hysterectomies, and tubal ligations by studying this phenomenon in countries and in time periods in which these operations were uncommon); this clearly suggests a protective effect of menopause (7). However, epidemiologic studies have not found this effect consistently. Although an increased risk of ovarian cancer in association with late age at menopause has been reported in a few studies, no association between age at natural menopause has been reported in most studies (see Schildkraut et al. [25] and references therein).

Why an effect of age at menopause is not consistently found is difficult to explain. It is possible that the difficulty may be because the early stages of ovarian cancer affect ovarian function and cause an early menopause. If this is so, then a more clear effect of age at menopause should be seen if one restricts attention to ovarian cancer patients diagnosed after 65 years of age, say. Such analyses have not been carried out and would be most informative. The lack of an effect of early menarche can, of course, be readily explained on an unopposed-E hypothesis because early menarche is associated with a more rapid onset of regular (ovulating)

cycles than a late menarche, so that amount of exposure to unopposed E may well be unaffected by age at menarche.

The current epidemiologic evidence obtained from cohort and population-based case–control studies with data on formulation and duration of use of menopausal hormone therapy (HT; 26–36) suggests that therapy with unopposed E increases ovarian cancer risk. Our meta-analysis of these studies finds an OR of approximately 24% per 5 years of use, with a 95% confidence interval of 13%–37% (37). The estimated overall risk associated with EPT use is smaller (13% per 5 years of use) and is not statistically significant. Some of this excess risk may be a diagnostic bias because we found that the ET effect was smaller when we restricted attention to regional or distant disease. A joint careful analysis of the published studies is needed to help clarify this issue.

17β-Estradiol has been found to increase ovarian cancer cell and ovarian cystadenoma cell proliferation in vitro (8), so the mechanism of any ET effect may be by direct stimulation of growth of the relevant premalignant or early malignant cells. Progesterone reduces cell proliferation under the same in vitro conditions, so one might predict that EPT would have a lesser effect on risk of ovarian cancer than ET. The relevance of these in vitro experiments is, however, open to some question because the doses of steroids used are much greater than those achieved with HT.

We saw a positive correlation between BMI and ovarian cancer risk in this study, and a recent systematic review (15) found a consistent positive association between body size and ovarian cancer risk in 11 population-based case–control studies and 5 cohort studies. Since that review, results from 2 other population-based case–control studies (38, 39) and 5 cohort studies (40–44) have been published. Two of these studies (41, 44) did not find such an effect; all the other studies did. The increase in risk between the upper and lower quartiles of BMI is around 40%. As we discussed above, we only saw this relationship with localized disease, and further investigation of this relationship is needed to determine its etiologic significance.

If BMI is related to ovarian cancer risk only incidentally, through increasing BMI being related to increased contact with the medical system, then this would cast some doubt on the increased incidence that is seen with ET use because BMI is associated with increased postmenopausal E levels and decreased sex hormone–binding globulin. One would therefore expect to see a clear association with BMI if the ET result was true.

Oral contraceptives block ovulation and the subsequent repair of the ovarian surface (the purported cell of origin of ovarian cancers). Rodriguez et al. (45, 46) have proposed, based on long-term studies in macaques of extended exposure to OCs or the individual components of OCs, that it is a direct action of progestins on OSE that provides the protection from OCs against ovarian cancer. In these studies, the progestin component of OCs, given alone without the E component, showed an increased apoptotic effect on the OSE that is very similar to that seen with OCs. This most interesting study is, however, difficult to interpret, because the progestin component alone would also block ovulation and follicle development, so that one cannot distinguish a direct progestin effect from an effect of suppression of follicle development and ovulation. This could be studied by using a GnRH analog to suppress ovulation.

What is the current situation regarding chemopreventive strategies against ovarian cancer? Oral contraceptive use remains an effective approach: in our data and in recently published studies, the protective effect was seen to be very long term, extending to women aged >60 years who had used OCs many years in the past. If the finding of a major protective effect of a late birth can be confirmed, then we may be able to exploit this for a short-term chemoprevention strategy with a long-term preventive effect. To do this, we will in all probability need to understand the mechanism of the protective effect. Because late incomplete pregnancies and late OC use do not appear to provide such an effect, we must look for the distinctive characteristics of a full-term pregnancy.

In vitro experiments on the effects of pregnancy levels of E and progesterone on ovarian tumor cells may be most informative. It is possible that the prolonged high levels of progesterone during a term pregnancy will be lethal to or will "terminally differentiate" a significant proportion of normal or "premalignant" ovarian cancer precursor cells. Observations on the ovaries of women immediately after delivery would be most informative, and this possibility could also be studied in macaques in a manner similar to that employed by Rodriguez et al. (45, 46) to study the effect of OCs.

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Exhibit 42

Risk Factors for Benign Serous and Mucinous Epithelial Ovarian Tumors

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OBJECTIVE: To investigate the risk factors for benign serous and mucinous epithelial ovarian tumors.

METHODS: Cases were women newly diagnosed with benign serous ovarian tumors (n=230) or benign mucinous tumors (n=133) between 2002 and 2005. Control women were selected at random from the general population (n=752). All participants completed a comprehensive reproductive and lifestyle questionnaire. Multivariable logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) and to simultaneously adjust for potential confounding factors.

RESULTS: Current smoking was associated with a threefold increase in risk of benign mucinous tumors (OR 3.25, 95% CI 1.97–5.34), and there was a trend of increasing risk with increasing amount smoked ($P<.001$). Both recent obesity (OR 1.93, 95% CI 1.30–2.88) and obesity at age 20 (OR 4.38, 95% CI 1.88–10.20) were associated with increased risk of benign serous ovarian tumors, and having had a hysterectomy was also related to increased risk of serous (OR 2.75, 95% CI 1.90–3.96), but not mucinous tumors. Ever having had a term pregnancy was inversely associated with both tumor types (combined OR 0.65, 95% CI 0.43–0.97), although greater numbers of pregnancies did not decrease risk further. Use of hormonal contraceptives was unrelated to risk.

CONCLUSION: Our results suggest some differences in risk factors between benign serous and mucinous epithelial ovarian tumors and that risk factors for benign serous tumors differ from those well established for ovarian cancer. The results also suggest that there is potential for prevention of these common conditions through avoidance of smoking and obesity.

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Benign epithelial ovarian tumors constitute a common gynecologic problem frequently requiring surgical treatment to alleviate symptoms and prevent complications. Although these tumors are diagnosed most often in women in their 30s or 40s, they can affect women of all ages and account for about 55% of all treated epithelial ovarian neoplasms.[1] The true incidence is, however, unknown. In the United States in 2002, approximately 44,000 female inpatients had a primary discharge diagnosis of a benign ovarian neoplasm[2] and total hospital treatment costs for this group are estimated at approximately $US 264 million. Although this figure includes an unknown proportion of nonepithelial tumors, it may underestimate the incidence of benign epithelial tumors, because many can be asymptomatic and remain undiagnosed and untreated.[3]

Current evidence suggests that benign serous tumors are unlikely to progress to high-grade serous cancer, although a precursor role in borderline ovarian cancer is possible.[4,5] In contrast, benign mucinous tumors seem to have the potential to progress through borderline tumors to invasive cancer.[4,5] Better understanding of the causes of benign tumors is needed, but only a few small studies have investigated this[6–9] and

rarely for serous and mucinous benign tumors separately.[9] Given the current lack of information regarding this common condition, we have conducted a comprehensive investigation of the risk factors for benign epithelial ovarian tumors, by histologic subtype.

MATERIALS AND METHODS

The study was based primarily in the Australian state of Queensland where women with benign epithelial ovarian tumors were identified in one of two ways. A number were identified by the Australian Ovarian Cancer Study (see below) and the remainder were ascertained through the single public and two major private pathology laboratories servicing Queensland. Between September 2003 and June 2005 the pathology laboratories conducted a monthly record search to identify all women aged 18 to 79 years newly diagnosed with a benign mucinous or serous ovarian tumor. Women who had not previously been recruited for Australian Ovarian Cancer Study were mailed study information and asked to indicate their willingness to be contacted about the study by returning a "permission to contact" form to the pathology laboratory. The details of those women indicating willingness to be contacted were then passed on to the study investigators who formally invited them to participate in the study. If the "permission to contact" form was not returned after two weeks, a second letter was sent.

The pathology laboratories approached 353 women in total. Of these, 74 (21%) did not respond to the mailed information and four were excluded due to poor health (n=2) or language problems (n=2). Among the remainder, further contact was refused by 40 women, and nine did not complete the questionnaire, giving an overall participation rate of 65%. Subsequent to review of the diagnostic pathology reports, a further six of these women were found to have ineligible pathology.

Additional women with benign ovarian tumors were identified through the Australian Ovarian Cancer Study, a national case–control study of ovarian cancer that recruited women between January 2002 and June 2005. Women with suspected ovarian cancer were invited to participate before surgical treatment and any subsequently found to have benign serous or mucinous ovarian tumors were included in the present study. An additional 55 women were identified in Queensland in this way, and eligible women were also included from the other Australian states: New South Wales (n=25), Victoria (n=21), South Australia (n=4), Western Australia (n=8) and Tasmania (n=25).

Two researchers independently abstracted information on site (ovary, fallopian tube, other), histologic subtype, and tumor behavior (benign, borderline, and invasive) from the pathology report of each case woman, and any discrepancies were resolved by consensus. For a sample of 87 women (including women with benign, borderline, and invasive tumors), the pathology reports and full set of diagnostic slides were formally reviewed by one of a group of gynecologic pathologists. The agreement between the results of the formal review and the abstracted data were 97% for tumor site, 98% for tumor behavior and 99% for histologic subtype. Only benign tumors of serous or mucinous subtype considered to have arisen from the ovary were included in the following analyses.

The comparison group for the present study was selected from the group of women who were recruited as controls for the Australian Ovarian Cancer Study. A computerized script was used to randomly select women from the Australian Electoral Roll (enrolment is compulsory in Australia) after frequency matching on age (in 5-year age bands) and state to the case series of women with ovarian cancer. Selected women were mailed an invitation letter and information brochure explaining the study and then, where possible, followed up by telephone. At least five attempts were made to reach each woman; those women who did not have a listed telephone number were mailed a second invitation letter. Because most women with benign tumors were from Queensland and in general were younger than women with ovarian cancer, we used a subset of the Australian Ovarian Cancer Study controls in the current analyses. We used SAS (SAS Institute Inc., Cary, NC) (proc surveyselect) to randomly select up to five Australian Ovarian Cancer Study controls for each woman with a benign epithelial tumor, after stratifying by state and age group (in 10-year age bands). Women were excluded as controls if they had a history of ovarian cancer or bilateral oophorectomy or (as for cases) if they were unable to provide informed consent or complete the study questionnaires due to language difficulties, illness, or mental incapacity. The overall participation rate for Australian Ovarian Cancer Study was 84% among cases and 47% among controls.

All participants gave consent and were asked to complete a health and lifestyle questionnaire, including questions relating to demographic and physical characteristics, family history, personal medical and surgical history, lifestyle habits (including smoking

and alcohol consumption), and reproductive and contraceptive histories. Missing information or inconsistencies were clarified during a subsequent telephone interview. A small number of women who did not return the full questionnaire completed an abbreviated questionnaire covering key exposures. Ethics approval was obtained from the Queensland Institute of Medical Research Human Research Ethics Committee and all relevant hospitals.

Effects were estimated by odds ratios (ORs) with 95% confidence intervals (CIs) calculated using unconditional multiple logistic regression to simultaneously adjust for potential confounding factors. Serous and mucinous tumors were initially examined separately, but because many of the associations were similar for serous and mucinous tumors, we have also reported results for the combined tumor group. To investigate linear trends for ordinal variables, the median value for each category was used in a continuous term, and the associated P value was assessed.[10] Stratified analyses were undertaken to investigate possible effect modification, and the statistical significance of potential interactions was assessed by including a multiplicative term in the models. Models were adjusted for age, hormonal contraceptive use, parity, education, hysterectomy status, and smoking status. Other potential confounding factors that changed the point estimates by less than 10% were not included in the final models. All statistical analyses were conducted using SAS 9.1 software (SAS Institute Inc.)

Use of combined oral, progestin-only, and injected (depot and implant formulations) contraceptives was combined for the analyses of the effects of hormonal contraceptives. Hormone replacement therapy included estrogen-only and combined estrogen–progestin regimes. For analyses of parity we included pregnancies of 6 or more months' duration; those lasting less than 6 months were considered to be incomplete pregnancies (including miscarriages, ectopic pregnancies, and induced abortions). Only women who had ever had a live birth were included in analyses of breastfeeding, and only those who had had a pregnancy of duration 6 or more months were included in analyses of the effect of age at first birth. The relation with obesity was assessed using measures of body mass index (BMI) calculated from self-reported height, weight at age 20, and weight 1 year before diagnosis (or first contact for controls) by dividing weight in kilograms by the square of height in meters. We used standard BMI categories for analysis (18.5–24.9 kg/m^2, "normal"; 25–29.9 kg/m^2, "overweight"; 30 kg/m^2 or more, "obese"). Risk associated with amount of smoking was assessed by calculating pack-years of smoking (the number of cigarettes smoked per day, multiplied by the number of years smoked, divided by 20). We estimated amount of perineal talc use by multiplying frequency of use by years of use. We excluded all exposures in the 12 months before diagnosis for cases (or first approach for controls) because we considered them unlikely to be associated with tumor causation.

RESULTS

The final case group included 225 women with benign serous tumors, 127 with benign mucinous tumors, and six women with one serous and one mucinous tumor. The latter group were included in analyses of both subtypes. Data from 754 control women were included. To ensure the 83 interstate women with benign ovarian tumors recruited through Australian Ovarian Cancer Study were not materially different from the population-based Queensland sample, we compared the groups across key variables. The interstate women were on average older than those from Queensland (57 years compared with 52 years, $P<.001$) reflecting the higher referral rate of older women to gynecologic oncology clinics, but the groups did not differ significantly with respect to parity, use of contraceptives, level of education, hysterectomy, tubal ligation, smoking status, or BMI (data not shown) and were therefore combined for analyses.

Women with benign mucinous tumors were, on average, younger than women with benign serous tumors (50 years compared with 55 years, $P=.003$). The average age of control women was 55 years. More than 95% of women were white, and ethnicity did not vary between the cases and controls.

We examined the relation between lifestyle and personal characteristics and risk of the different tumor types (Table 1). Level of education, as a marker of socioeconomic status, was not significantly related to risk of either tumor type. Smoking status was, however, strongly and significantly associated with the risk of benign tumors, both mucinous and serous (OR 3.3, 95% CI 2.0–5.3 and OR 2.3, 95% CI 1.5–3.5, respectively, for current smokers compared with never smokers). Significant trends of increasing risk with increasing pack-years of smoking were observed for both tumor types, although the test for linear trend suggested a closer association with mucinous tumors (P for trend=.001) than serous tumors (P for trend=.02).

Obesity (BMI 30 or more) at age 20 years and at 1 year before diagnosis was related to an increased

Table 1. Adjusted* Odds Ratios and 95% Confidence Intervals for the Association Between Selected Personal Characteristics and Lifestyle Factors and Risk of Benign Mucinous and Serous Ovarian Tumors

	Controls (n=752)	Mucinous (n=133)	Serous (n=230)	Mucinous	Serous	Combined
Level of education						
School only	382 (51)	69 (52)	114 (49)	1.00	1.00	1.00
Technical	270 (36)	44 (33)	87 (38)	0.86 (0.55–1.34)	1.10 (0.78–1.60)	0.99 (0.73–1.33)
University	100 (13)	20 (15)	29 (13)	0.94 (0.50–1.73)	1.09 (0.65–1.84)	1.00 (0.65–1.54)
Smoking status						
Never	471 (63)	61 (46)	116 (51)	1.00	1.00	1.00
Ex-smoker	200 (26)	31 (23)	64 (28)	1.36 (0.84–2.21)	1.22 (0.84–1.76)	1.29 (0.94–1.77)
Current	81 (11)	41 (31)	48 (21)	3.25 (1.97–5.34)	2.26 (1.45–3.53)	2.60 (1.79–3.78)
Pack-years of smoking						
None	471 (63)	61 (54)	116 (59)	1.00	1.00	1.00
10 or less	139 (18)	10 (9)	28 (14)	0.53 (0.26–1.09)	0.73 (0.45–1.17)	0.65 (0.43–0.99)
Less than 10 to less than 20	44 (6)	13 (12)	15 (8)	2.13 (1.03–4.38)	1.29 (0.67–2.50)	1.58 (0.92–2.69)
20 to less than 30	39 (5)	16 (14)	16 (8)	3.42 (1.73–6.75)	1.61 (0.84–3.09)	2.26 (1.33–3.82)
30 or more	55 (7)	13 (12)	21 (11)	2.31 (1.15–4.65)	1.75 (0.98–3.13)	1.97 (1.21–3.20)
				P for trend<.001	*P* for trend=.02	*P* for trend<.001
Body mass index 1 year ago						
Less than 25	335 (46)	48 (39)	85 (40)	1.00	1.00	1.00
25 or more to 30	230 (32)	44 (36)	55 (26)	1.56 (0.97–2.52)	1.08 (0.72–1.61)	1.26 (0.91–1.76)
30 or more	158 (22)	31 (25)	73 (34)	1.43 (0.83–2.44)	1.93 (1.30–2.88)	1.69 (1.19–2.40)
				P for trend=.1	*P* for trend=.002	*P* for trend=.003
Body mass index at age 20 y						
Less than 25	660 (91)	107 (85)	183 (84)	1.00	1.00	1.00
25 or more to 30	53 (7)	12 (9)	21 (9)	1.06 (0.51–2.18)	1.24 (0.71–2.19)	1.12 (0.68–1.83)
30 or more	15 (2)	7 (6)	15 (7)	2.50 (0.85–7.31)	4.38 (1.88–10.2)	3.31 (1.55–7.07)
				P for trend=.2	*P* for trend=.002	*P* for trend=.007
Age at menarche (y)						
12 or younger	283 (39)	55 (42)	97 (42)	1.00	1.00	1.00
13	211 (29)	42 (32)	58 (25)	1.11 (0.69–1.79)	0.79 (0.52–1.18)	0.91 (0.65–1.27)
14 or older	236 (32)	33 (26)	74 (32)	0.79 (0.48–1.30)	0.97 (0.66–1.42)	0.88 (0.64–1.22)
				P for trend=.9	*P* for trend=.4	*P* for trend=.7
Use of talc in the perineal region						
No	419 (56)	74 (56)	126 (55)	1.00	1.00	1.00
Yes	332 (44)	59 (44)	103 (45)	1.19 (0.80–1.76)	1.04 (0.75–1.43)	1.10 (0.84–1.45)
Amount of talc used in the perineal region						
None	419 (56)	76 (57)	126 (55)	1.00	1.00	1.00
Minimal	74 (10)	14 (11)	14 (6)	1.02 (0.53–1.98)	0.70 (0.37–1.30)	0.85 (0.52–1.38)
Moderate	86 (11)	18 (14)	21 (9)	1.57 (0.87–2.84)	0.85 (0.49–1.48)	1.05 (0.68–1.64)
Substantial	161 (21)	24 (18)	61 (27)	0.98 (0.58–1.66)	1.21 (0.82–1.79)	1.16 (0.83–1.62)
				P for trend=.9	*P* for trend=.2	*P* for trend=.3
Family history of breast or ovarian cancer in a first-degree relative						
No	647 (86)	120 (90)	194 (84)	1.00	1.00	1.00
Yes	105 (14)	13 (8)	36 (16)	0.73 (0.38–1.38)	1.27 (0.82–1.97)	1.11 (0.75–1.63)

Data are n (%) or odds ratio (95% confidence interval). Numbers in first three columns may not add to total due to missing values.
* Adjusted for age (younger than 40,40–49, 50–59, 60–69, 70 years or older), state of residence, education (school only, technical, university), parity (0, 1–2, 3 or more), hormonal contraceptive use (never, less than 5years use, 5 years or more use), hysterectomy (yes or no), smoking status (never, ex-, current).

risk of both tumor types, but significantly so only for serous tumors (OR 4.4, 95% CI 1.9–10.2 compared with normal BMI at age 20 and OR 1.9, 95% CI 1.3–2.9 compared with normal BMI 1 year ago). Overall, there was a significant trend of increasing risk of serous tumors with increasing BMI at both time points (P=.002) but not for mucinous tumors (P=.1 for BMI 1 year ago, and P=.2 for BMI at age 20). When we stratified by age (50 years or younger compared with more than 50 years), the association between recent obesity and serous tumors was stronger for those 50 years or younger at diagnosis (OR

2.4, 95% CI 1.1–4.9) than among those over 50 (OR 1.6, 95% CI 1.0–2.7), although the difference with age was not statistically significant. Neither age at menarche nor a family history of breast or ovarian cancer nor use of talc in the perineal region were significantly related to risk of either tumor type (Table 1).

Women who reported at least one term pregnancy had a nonsignificant 40% reduction in risk of mucinous tumors and a 38% reduction in risk of serous tumors (Table 2). No clear trend of decreasing risk with increasing numbers of term pregnancies was seen for either subtype, although the odds ratio was less than 1 for all categories of parity. In contrast, increasing numbers of incomplete pregnancies were associated with a significantly increased risk of mucinous tumors (OR 1.8, 95% CI 1.1–3.1 for two or more compared with none, *P* for trend was .006), but did not seem to be associated with serous tumors. Having a first term pregnancy at less than 20 years of age was associated with a small increase in risk of serous tumors (OR 1.6, 95% CI 1.0–2.4 for age of first birth less than 20 years compared with greater than 20 years), but there was no associated trend for either tumor type. Age at last birth (data not shown) and breastfeeding were not significantly related to risk of serous or mucinous benign tumors.

Ever use of hormonal contraceptives was not associated with risk of either serous (OR 1.3, 95% CI 0.8–2.0) or mucinous benign tumors (OR 1.1, 95% CI 0.6–1.9). Although not statistically significant, there seemed to be somewhat different associations with disease depending upon the duration of use. There was a suggestion that very long-term use (more than 15 years) might decrease the risk of both tumor types compared with no use, (OR 0.7, 95% CI 0.3–1.5 for mucinous tumors and OR 0.6, 95% CI 0.3–1.2 for serous), whereas the odds ratios were generally above unity for shorter durations of use (Table 3). Too few women had used long-acting progestins to independently assess their relation with tumor occurrence, but

Table 2. Adjusted* Odds Ratios and 95% Confidence Intervals for the Association Between Pregnancy-Related Factors and Risk of Benign Mucinous and Serous Ovarian Tumors

	Controls	Mucinous	Serous	Mucinous	Serous	Combined
Parous						
No	81 (11)	28 (21)	33 (14)	1.00	1.00	1.00
Yes	671 (89)	105 (79)	197 (86)	0.60 (0.35–1.05)	0.72 (0.44–1.18)	0.65 (0.43–0.97)
Parity						
0	81 (11)	28 (21)	33 (14)	1.00	1.00	1.00
1	71 (9)	11 (8)	28 (12)	0.43 (0.19–0.98)	0.97 (0.51–1.83)	0.70 (0.40–1.21)
2	243 (32)	37 (28)	64 (28)	0.58 (0.31–1.08)	0.68 (0.40–1.18)	0.62 (0.39–0.96)
3	210 (28)	31 (23)	54 (24)	0.65 (0.33–1.25)	0.62 (0.35–1.09)	0.61 (0.38–0.97)
4 or more	147 (20)	26 (20)	50 (22)	0.85 (0.42–1.72)	0.74 (0.41–1.34)	0.75 (0.45–1.24)
				P for trend=.9	*P* for trend=.7	*P* for trend=.9
Incomplete pregnancies†						
0	486 (65)	77 (58)	139 (61)	1.00	1.00	1.00
1	169 (22)	29 (22)	59 (26)	1.11 (0.67–1.84)	1.19 (0.82–1.73)	1.18 (0.86–1.63)
2 or more	97 (13)	27 (20)	31 (13)	1.84 (1.08–3.14)	0.96 (0.59–1.57)	1.19 (0.80–1.76)
				P for trend=.04	*P* for trend=.6	*P* for trend=.2
Breastfeeding duration (mo)‡						
Never	110 (17)	20 (20)	33 (18)	1.00	1.00	1.00
6 or less	159 (24)	26 (26)	49 (26)	1.04 (0.52–2.06)	1.05 (0.61–1.81)	1.05 (0.66–1.67)
7–12	123 (19)	16 (16)	39 (21)	0.87 (0.40–1.92)	1.18 (0.66–2.10)	1.00 (0.60–1.65)
More than12	266 (40)	39 (38)	64 (35)	1.00 (0.50–1.99)	0.92 (0.53–1.59)	0.91 (0.57–1.45)
				P for trend= 1.00	*P* for trend=.6	*P* for trend=.5
Age at first term birth (y)§						
Less than 20	91 (14)	20 (20)	47 (25)	1.00	1.00	1.00
20–24	309 (47)	46 (47)	73 (40)	0.89 (0.48–1.66)	0.59 (0.37–0.94)	0.64 (0.42–0.96)
25–29	180 (27)	24 (25)	44 (24)	0.88 (0.43–1.80)	0.64 (0.37–1.09)	0.67 (0.42–1.07)
More than 30	80 (12)	8 (8)	21 (11)	0.64 (0.24–1.71)	0.70 (0.35–1.40)	0.63 (0.35–1.16)
				P for trend=.4	*P* for trend=.4	*P* for trend=.2

Data are n (%) or odds ratio (95% confidence interval). Numbers in first three columns may not add to total due to missing values.

* Adjusted for age (younger than 40, 40–49, 50–59, 60–69, 70 years or older), state of residence, education (school only, technical, university), hormonal contraceptive use (never, less than 5 years use, 5 years use or more), hysterectomy (yes or no), smoking status (never, ex-, current).

† Additionally adjusted for parity (0, 1–2, 3 or more).

‡ Among women who have had a live birth. Additionally adjusted for number of live births.

§ Among women who have had a term birth. Additionally adjusted for parity (0, 1–2, 3 or more).

Table 3. Adjusted* Odds Ratios and 95% Confidence Intervals for the Association Between Exogenous Hormone Use and Risk of Mucinous and Serous Benign Ovarian Tumors

	Controls	Mucinous	Serous	Mucinous	Serous	Combined
Duration hormonal contraceptive use (mo)						
Never	168 (22)	22 (17)	41 (18)	1.00	1.00	1.00
1–12	64 (9)	12 (9)	22 (10)	1.16 (0.51–2.65)	1.30 (0.69–2.46)	1.24 (0.72–2.14)
13–60	153 (20)	35 (27)	59 (26)	1.29 (0.68–2.46)	1.41 (0.85–2.33)	1.32 (0.86–2.03)
61–120	146 (19)	26 (20)	54 (24)	1.00 (0.51–1.99)	1.46 (0.88–2.44)	1.28 (0.82–1.98)
121–180	111 (15)	23 (17)	31 (13)	1.06 (0.52–2.15)	1.06 (0.59–1.88)	1.08 (0.67–1.74)
More than 180	109 (15)	14 (10)	21 (9)	0.69 (0.32–1.53)	0.64 (0.34–1.19)	0.69 (0.41–1.16)
				P for trend=.2	*P* for trend=.06	*P* for trend=.06
Duration of hormone replacement therapy use (y)						
Never	476 (64)	98 (74)	151 (67)	1.00	1.00	1.00
Less than 2	63 (8)	14 (11)	15 (7)	1.35 (0.67–2.70)	0.67 (0.35–1.29)	0.90 (0.54–1.49)
2 to 5	65 (9)	4 (3)	18 (8)	0.39 (0.13–1.12)	0.82 (0.45–1.52)	0.70 (0.41–1.22)
More than 5 to 10	62 (8)	9 (7)	17 (7)	0.99 (0.45–2.20)	0.70 (0.37–1.31)	0.80 (0.47–1.37)
More than 10	79 (11)	7 (5)	25 (11)	0.67 (0.28–1.64)	0.71 (0.40–1.27)	0.70 (0.42–1.17)
				P for trend=.2	*P* for trend=.3	*P* for trend=.2

Data are n (%) or odds ratio (95% confidence interval). Numbers in first three columns may not add to total due to missing values.
* Adjusted for age (younger than 40, 40–49, 50–59, 60–69, 70 years or older), state of residence, education (school, technical, university), parity (0, 1–2, 3 or more), hysterectomy (yes or no), smoking status (never, ex-, current).

excluding women who had used these from the analyses did not substantially change the odds ratios associated with hormonal contraceptive use. The use of hormone replacement therapy was inversely associated with risk of serous tumors (OR 0.7, 95% CI 0.5–1.1 for ever compared with never use) but there was no trend of further decrease with longer duration of use (*P* for trend=.3). Mucinous tumors had no apparent relation with hormone replacement therapy use (OR 0.9, 95% CI 0.5–1.5 for ever compared with never use, *P* for trend=.2)

We found no evidence that tubal ligation was associated with risk of either mucinous or serous benign tumors, and hysterectomy was unrelated to risk of mucinous tumors (Table 4). However, having a hysterectomy was associated with an almost three-fold increase in risk of benign serous tumors (OR 2.8, 95% CI 1.9–4.0). We also examined this in relation to indication for hysterectomy. If the reason for hysterectomy was a nonhormonal condition such as prolapse or cervical dysplasia, then the risk for serous tumors was not increased (OR 1.1, 95% CI 0.5–2.7). However, if the indication for hysterectomy was a hormonally responsive condition such as endometriosis, menorrhagia, or adenomyosis, then the associated risk for serous tumors increased three-fold (OR 3.0, 95% CI 2.1–4.5).

A self-reported history of endometriosis or fibroids was unrelated to benign tumor risk. However, being diagnosed with polycystic ovary syndrome (PCOS) was associated with nonsignificant increases in risk of both mucinous and serous tumors (OR 1.7, 95% CI 0.6–5.3 for mucinous tumors and OR 2.2, 95% CI 0.9–5.5 for serous tumors). Further adjustment of this association for BMI at age 20 years weakened the association with mucinous tumors (OR 1.1, 95% CI 0.3–3.8), but if anything strengthened the relation with serous tumors (OR 2.3, 95% CI 0.9–5.9). Reported history of abnormal Pap tests, genital warts, genital herpes, pelvic inflammatory disease (*Chlamydia*), or other sexually transmitted infections were unrelated to either tumor type (data not shown).

DISCUSSION

The effect of reproductive risk factors on serous and mucinous tumors in our study were similar and largely in keeping with previous studies.[7–9,11] Term pregnancy was associated with a decreased risk of both tumor types, but increasing parity did not seem to confer additional protection, and use of hormonal contraceptives and breastfeeding were not associated with risk. This is in stark contrast to invasive epithelial ovarian cancer, which is strongly inversely associated with these exposures.[12–14]

The association we observed with smoking was more marked for mucinous than serous benign tumors, similar to the associations reported for ovarian cancer.[15,16] In addition, we found body mass index, both recent and at age 20, to be more strongly related to serous than mucinous tumors. Hysterectomy and PCOS were also associated with the risk of serous but not mucinous tumors.

Table 4. Adjusted* Odds Ratios and 95% Confidence Intervals for the Association Between Medical Procedures and Gynecologic Conditions and Risk of Mucinous and Serous Benign Ovarian Tumors

	Controls	Mucinous	Serous	Mucinous	Serous	Combined
Tubal sterilization						
No	532 (71)	100 (75)	162 (70)	1.00	1.00	1.00
Yes	219 (29)	33 (25)	68 (30)	1.00 (0.61–1.64)	1.08 (0.75–1.57)	1.04 (0.76–1.44)
Hysterectomy						
No	610 (81)	112 (84)	147 (64)	1.00	1.00	1.00
Yes	142 (19)	21 (16)	83 (36)	0.95 (0.55–1.67)	2.75 (1.90–3.96)	1.91 (1.38–2.66)
Endometriosis						
No	705 (94)	117 (89)	188 (84)	1.00	1.00	1.00
Yes	44 (6)	15 (11)	36 (16)	1.06 (0.47–2.43)	1.24 (0.69–2.23)	1.16 (0.68–1.98)
PCOS						
No	736 (98)	124 (94)	212 (95)	1.00	1.00	1.00
Yes	13 (2)	8 (6)	12 (5)	1.71 (0.56–5.25)	2.18 (0.87–5.48)	1.83 (0.81–4.13)
Fibroids						
No	617 (82)	106 (80)	149 (66)	1.00	1.00	1.00
Yes	134 (18)	27 (20)	76 (34)	1.10 (0.62–1.97)	1.36 (0.91–2.05)	1.31 (0.91–1.88)
Abnormal pap test						
No	609 (81)	93 (74)	170 (78)	1.00	1.00	1.00
Yes	141 (19)	32 (26)	49 (22)	1.28 (0.78–2.09)	1.19 (0.80–1.78)	1.18 (0.84–1.67)

PCOS, polycystic ovary syndrome.
Data are n (%) or odds ratio (95% confidence interval). Numbers in first three columns may not add to total due to missing values.
* Adjusted for age (younger than 40, 40–49, 50–59, 60–69, 70 years or older), state of residence, education (school, technical, university), parity (0, 1–2, 3 or more), hormonal contraceptive use (never, less than 5 years use, 5 years use or more), hysterectomy (yes or no), smoking status (never, ex-, current).

Population attributable risk percents estimated using these results suggest that approximately 20% of all benign mucinous tumors could be attributed to smoking and 17% of benign serous tumors could be attributed to obesity. If the incidence of benign epithelial tumors is 44,000 per year in the United States, and assuming that mucinous tumors comprise one third and serous tumors two thirds of those, then our results suggest that approximately 8,000 benign epithelial tumors could be prevented each year if smoking and obesity could be eliminated in the population.

Considering potential sources of error in our results, the response rates among both cases and controls were less than optimal at 65% and 47%, respectively, raising the possibility that women who took part differed systematically from those who did not. To assess this we compared our control group's responses to data from the 2001 Australian National Health Survey (NHS), with a response rate of approximately 90%.[17] Distributions of parity and body mass index among our control women were almost identical to those surveyed in the NHS. Prevalence of use of the oral contraceptive in women aged younger than 50 years was approximately 5% higher among our controls than women in the NHS, suggesting that any inverse association with long-term use is even weaker than observed. The age-standardized rate of hysterectomy in our controls was slightly lower than in the NHS (19% compared with 24%), and if true, then the magnitude of the observed risk of serous tumors associated with hysterectomy may have been overestimated, but sensitivity analyses suggested that this could not fully explain the elevated risk. This underestimation could also have masked a stronger inverse association with mucinous tumors. Finally, the prevalence of current smoking was lower among our controls compared with the NHS women (11% compared with 17%). Sensitivity analyses suggest that this difference could account for much of the apparent increase in risk of serous tumors associated with current smoking, but is insufficient to explain the stronger association observed for mucinous tumors (although the true magnitude of the association may be somewhat lower than that observed) (personal communication, N. Pandeya, research scholar, Queensland Institute of Medical Research, August 2006).

An appropriate comparison population for our case group is not available, but given that surgery is curative for this condition, it seems likely that response patterns in this group are not dissimilar to those of unaffected women. Accordingly, higher levels of smoking would be expected in the nonresponders,[18,19] whereas distributions of BMI are likely to be similar among responders and nonresponders.[18,20] Thus, the suboptimal response is unlikely to distort our main findings, and, in practice,

such nondifferential error would mean that the true associations might be stronger than those observed. Although the possibility of confounding cannot entirely be dismissed, it is not likely to be a major source of error here, because results adjusted for key factors differed little from results adjusted for age only.

Finally detection bias would not explain the association between benign tumors and PCOS and hysterectomy because associations were limited to serous tumors and because increased medical surveillance of women with other gynecologic conditions such as abnormal Pap tests and genital herpes yielded no similar significant associations with benign ovarian tumors.

While the results of our study do not suggest a strong link between pregnancy, hormonal contraceptives, or breastfeeding and benign serous tumors, they do suggest a possible causal role for sex steroid hormones. Hysterectomy performed for hormonally responsive conditions was related to increased risk of serous tumors, whereas PCOS and obesity, which have well-documented effects on sex steroids and insulin,[21] were also associated with serous tumor occurrence. The observed associations might therefore implicate androgens or insulin in the pathogenesis of these tumors. Because adjustment for BMI did not substantially change the relation between serous tumors and PCOS, our results suggest independent effects of PCOS and obesity.

In conclusion, we have found that parity is associated with a modestly decreased risk of both serous and mucinous benign ovarian tumors, but increasing parity does not further decrease risk. Obesity, PCOS, and hysterectomy were related to increased risk of serous tumors, whereas smoking was strongly associated with the occurrence of mucinous tumors. These results require replication but suggest that along with many other conditions, a reduction in smoking and obesity could help prevent women from developing benign serous and mucinous ovarian tumors and thus could ultimately lessen the effect of these relatively common conditions on modern gynecologic practice.

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Exhibit 43

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Talcum powder, chronic pelvic inflammation and NSAIDs in relation to risk of epithelial ovarian cancer

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Chronic inflammation has been proposed as the possible causal mechanism that explains the observed association between certain risk factors, such as the use of talcum powder (talc) in the pelvic region and epithelial ovarian cancer. To address this issue we evaluated the potential role of chronic local ovarian inflammation in the development of the major subtypes of epithelial ovarian cancer. Factors potentially linked to ovarian inflammation were examined in an Australia-wide case–control study comprising 1,576 women with invasive and low malignant potential (LMP) ovarian tumours and 1,509 population-based controls. We confirmed a statistically significant increase in ovarian cancer risk associated with use of talc in the pelvic region (adjusted odds ratio 1.17, 95% CI: 1.01–1.36) that was strongest for the serous and endometrioid subtypes although the latter was not statistically significant (adjusted odds ratios 1.21, 95% CI 1.03–1.44 and 1.18, 95% CI 0.81–1.70, respectively). Other factors potentially associated with ovarian inflammation (pelvic inflammatory disease, human papilloma virus infection and mumps) were not associated with risk but, like others, we found an increased risk of endometrioid and clear cell ovarian cancer only among women with a history of endometriosis. Regular use of aspirin and other nonsteroidal anti-inflammatory drugs was inversely associated with risk of LMP mucinous ovarian tumours only. We conclude that on balance chronic inflammation does not play a major role in the development of ovarian cancer.

Key words: ovarian cancer; chronic inflammation; talcum powder

Chronic inflammation (hereafter referred to as inflammation) was first invoked as a possible mechanism leading to the development of epithelial ovarian cancer to explain observed associations between certain factors, such as use of talcum powder in the perineal region or pelvic inflammatory disease (PID) and risk of ovarian cancer.[1] The major mechanisms thought to underlie ovarian carcinogenesis, namely increased pituitary gonadotropins or incessant ovulation, do not explain such associations.

A link between inflammation and cancer in general has long been recognized. As early as 1863, Virchow noticed the presence of leukocytes in cancer tissues and suggested a possible connection between inflammation and cancer.[2] Since inflammation also represents the process by which the immune system responds to infection or irritation, however, it has been referred to as a 'double-edged sword' with acute (beneficial) inflammation distinguished from the chronic (detrimental) inflammation that may prevent a robust anti-tumour response.[3]

Indeed the most consistent evidence linking inflammation with ovarian cancer comes from the many reports that use of talc in the perineal region increases ovarian cancer risk.[4,5] It has been suggested that the association between talc use and ovarian cancer is strongest for serous tumours when compared to other less common subtypes.[4,6,7] This would be consistent with the histological similarities observed between serous ovarian cancer and mesothelioma, which is known to be caused by asbestos, and the shared

Abbreviations: ACS, Australian Cancer Study; AOCS, Australian Ovarian Cancer Study; BMI, body mass index; HPV, human papilloma virus; LMP, low malignant potential; NSAIDs, non-steroidal anti-inflammatory drugs; OC, oral contraceptive; PID, pelvic inflammatory disease; STI, sexually transmitted infection.

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chemical properties of talcum powder and asbestos. Testing various factors that are possibly related to ovarian inflammation in a case–control study, Ness *et al.*[8] found that perineal talc use and endometriosis, defined as the presence of endometrial tissue outside the uterus and associated with localised inflammation at the site of endometriotic implants, were positively associated with ovarian cancer risk. However, they saw no association with PID, which they had also expected to be associated with increased risk.[8] Extending these epidemiological analyses, McSorley *et al.*[9] recently found significantly higher circulating C-reactive protein (CRP) levels, a marker of systemic chronic inflammation, among 167 women with incident ovarian cancer risk in a multicentre nested case–control study.

The potential role of ovarian inflammation in the development of ovarian cancer remains an open question. The aim of the current study was to further examine the role of local chronic inflammation in the development of epithelial ovarian cancer overall and by histologic subtype. In addition to talcum powder use, we examined medical conditions that cause inflammation in the pelvic region, including endometriosis and PID, and we also tested the hypothesis that if inflammation causes ovarian cancer then regular use of anti-inflammatory drugs should be inversely associated with this disease.

Material and methods

Study design

The Australian Ovarian Cancer Study is an Australia-wide population-based case–control study of epithelial ovarian cancer. It includes incident cases of invasive and low malignant potential (LMP) ovarian cancer diagnosed in women (aged 18–79 years) between January 2002 and June 2005. A total of 3,553 women were identified with suspected ovarian cancer. Of these, 304 died before contact could be made, physicians refused to give consent to contact 133, usually because they were too sick or unable to give informed consent and 194 women could not be contacted. A further 167 (5%) were excluded on the basis of language difficulties (70), mental incapacity (33) and illness (64). The remaining 2,755 women were invited to participate and, of these, 2,319 (84% of those approached) agreed to take part.

Two researchers independently abstracted information on tumour site, histological subtype and tumour behaviour (invasive *vs.* LMP) from the diagnostic histopathology reports and discrepancies were resolved by consensus. For a sample of 87 women, the pathology reports and full set of diagnostic slides were reviewed by a gynaecologic pathologist and the agreement with the original abstracted data was more than 97% for tumour site, behaviour and subtype. After histopathology review, 624 women were excluded because they were found to have nonepithelial, nonovarian or benign tumours and 10 because their cancer was first diagnosed before the start of the study period. Of the final 1,685 eligible participants with invasive or LMP cancers of the ovary, peritoneum or fallopian tube, 1,576 (94%) returned a questionnaire and comprised the case population in the current study. Separate analyses were also carried out for the 994 serous, 191 mucinous, 141 endometrioid and 88 clear cell tumours (the remaining 162 tumours were of other epithelial or mixed subtypes).

Potential control participants were identified from the Australian Electoral Roll (all citizens are required by law to enrol). Controls were frequency-matched to the entire case series based on age (5-year groups) and state of residence. In all, 3,600 women were contacted. Of these, 158 were ineligible because of language difficulties ($n = 97$) or illness ($n = 61$) and 16 were unable to be contacted a second time. Of the 3,426 eligible women, 1,612 (47%) agreed to participate and returned a questionnaire. From these women, 6 were excluded because they reported a previous ovarian cancer and 97 because of a previous bilateral oophorectomy resulting in a total of 1,509 controls for study.

Study participants filled in a comprehensive health and lifestyle questionnaire, which included questions about their personal details, physical characteristics, family history, medical and surgical history, lifestyle habits and reproductive factors. To determine use of talcum powder in the perineal region, participants were asked whether they had ever used powder or talc in the genital area or on underwear or sanitary pads/diaphragm. They were asked their age at first use and years of talc use in these areas. Duration of talcum powder use prior to and after hysterectomy/tubal ligation was calculated and in all analyses perineal talc use was defined as use occurring while the reproductive tract was patent (*i.e.*, prior to hysterectomy/tubal ligation for those women who had undergone gynaecological surgery). Information on talc use under the arms or on the chest or abdomen was also collected.

To measure use of nonprescription anti-inflammatory medications, participants were given examples of the type of medication (*e.g.*, aspirin) followed by a list of the common generic and brand names. To quantify the frequency of use, participants were asked how often they had taken various medications over the past 5 years (ranging from never to as much as twice or more per day). The current analyses were restricted to medications known to suppress inflammation namely aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). Participants were also asked whether they had ever had any of a number of specific medical conditions and, if so, the ages at which these were diagnosed.

Ethics approval was received from the Human Research Ethics Committees at the Queensland Institute of Medical Research, Peter MacCallum Cancer Centre, University of Melbourne, all participating hospitals and cancer registries.

Statistical analysis

Risk estimates were calculated as odds ratios (OR) with 95% confidence intervals (CI). χ^2-Squared tests were used to test for differences in patient characteristics (*e.g.*, age, level of education). All significance tests were 2-sided and a p-value of less than 0.05 was taken as significant. Unconditional multiple logistic regression models were constructed to simultaneously adjust for confounding factors.

Exposures to factors of interest occurring in the 12 months prior to diagnosis for cases (or 12 months prior to first contact for controls) were excluded because the aetiological influence of very recent exposures on incident ovarian cancer is likely to be minimal and, in cases, recent behaviours may reflect the presence of subclinical disease. All models were adjusted for the categorical variables of age in 10-year groups (<50, 50–59, 60–69, ≥70), highest level of education, parity (number of pregnancies >6 months) and duration of contraceptive use (including oral contraceptive pills and contraceptive injections). Analyses of endometriosis and potential symptoms of endometriosis (painful or long periods) were also adjusted for the categorical variable of body mass index (BMI) 1 year prior to diagnosis/recruitment (≤24.9, 25–29.9, ≥30 kg/m^2). Other potential confounders that were considered for all analyses but not included in the final models since they did not substantially alter risk estimates were: income, family history of ovarian or breast cancer, hysterectomy and/or tubal ligation and smoking.

All analyses were performed using the SAS system V 9.1 (SAS Institute, Cary, NC). Tests for linear trend were performed using the maximum likelihood test with the categorical variable of interest entered as a continuous term.

Results

The final study population included 1,576 women with epithelial ovarian cancer (invasive and LMP) and 1,509 controls. Cases were significantly older than controls (mean age cases = 57.8, mean age controls = 56.42, $p = 0.001$) and were less likely to have continued their education beyond high school (Table I). As expected, cases were significantly more likely to be nulliparous

TABLE I – DESCRIPTIVE CHARACTERISTICS OF 1,576 WOMEN WITH EPITHELIAL OVARIAN CANCER AND 1,509 RANDOMLY SELECTED POPULATION-BASED CONTROLS

Variable	Controls[1] (*N* = 1,509) *N* (%)	Cases[1] (*N* = 1,576) *N* (%)	*p*-Value
Highest level of education			
High school	735 (49)	851 (54)	0.02[2]
Technical college/ trade certificate	550 (37)	502 (32)	
University	218 (15)	214 (14)	
Number pregnancies (≥6 months)			
Nulliparous	181 (12)	298 (19)	<0.0001[3]
1–2	644 (43)	647 (41)	
≥3	684 (45)	628 (40)	
Ever used oral contraceptives			
No	330 (22)	505 (32)	<0.0001[3]
≤5 years	361 (24)	432 (28)	
>5 years	811 (54)	619 (40)	
Previous tubal ligation	406 (27)	355 (23)	0.0003[2]
Previous hysterectomy	289 (19)	364 (23)	0.05[2]
Mother/sister with ovarian or breast cancer	195 (13)	273 (19)	0.002[2]

[1]Numbers may not sum to total because of missing data.–[2]χ^2-square test for heterogeneity, adjusted for age group (10 year categories).–[3]χ^2-square test for trend, adjusted for age group (10 year categories).

and to report a mother or sister with ovarian or breast cancer. Cases were less likely to have used oral contraceptives or to report a previous tubal ligation. Unexpectedly, cases were somewhat more likely to report a prior hysterectomy (Table I).

Ever use of talc in the perineal region (among women with patent fallopian tubes) was associated with a significant increase in risk of all types of epithelial ovarian cancer combined (adjusted OR = 1.17, 95% CI: 1.01–1.36) (Table II). Analysis by histological subtype showed that the increase in risk was strongest for serous and endometrioid tumours although it was only statistically significant for serous tumours (adjusted OR = 1.21, 95% CI: 1.03–1.44 and 1.18, 95% CI 0.81–1.70, respectively). This increased risk was seen for both invasive and LMP serous tumours (data not shown), although the association with LMP tumours was not statistically significant because of the smaller numbers. There was no clear trend of increasing risk with longer duration of use, although tests for trend were of borderline statistical significance for all cancers and the serous subgroup (p = 0.02 for both). When we considered invasive and LMP tumours separately, a modest but statistically significant increase in risk of invasive serous tumours was observed in the highest category of use (over 25 years, adjusted OR = 1.35, 95% CI: 1.06–1.72), whereas little or no increased risk was observed with less than 25 years of use. For serous LMP tumours, a modest increase in risk was observed only in the lowest duration of use category (upto 10 years, adjusted OR = 1.71, 95% CI: 1.07–2.73) with no association for over 10 years of use.

Increased risk of ovarian cancer was specifically related to talc use in the pelvic region as talc use on other body sites showed no association (OR = 1.01, 95% CI: 0.84–1.20). In contrast to the elevated risk of ovarian cancer observed with perineal talc use prior to hysterectomy and/or tubal ligation, talc use after such surgery showed no association with serous ovarian cancer risk, regardless of duration (Table II).

Prior to 1976, talcum powder was often contaminated with asbestos fibres.[10,11] To assess whether the association between use of talc and ovarian cancer risk varied over time we evaluated this separately for different age groups. Our assumption was that use of talcum powder among older women would largely have been prior to 1976 (when voluntary guidelines to prevent asbestos contamination of talcum powder were adopted) whereas a greater pro-

TABLE II – ASSOCIATION BETWEEN PERINEAL TALCUM POWDER USE (SEPARATING THE EFFECTS OF USE PRIOR TO AND AFTER HYSTERECTOMY AND/OR TUBAL LIGATION) AND RISK OF EPITHELIAL OVARIAN CANCER

	Controls[1] *N* (%)	All cases[1] *N* (%)	All cases (*N* = 1,576) OR[2] (95% CI)	Serous (*N* = 994) OR[2] (95% CI)	Mucinous (*N* = 191) OR[2] (95% CI)	Endometrioid (*N* = 141) OR[2] (95% CI)	Clear cell (*N* = 88) OR[2] (95% CI)
Perineal use of talcum powder[3]							
Never	835 (57)	821 (54)	1.0	1.0	1.0	1.0	1.0
Ever	635 (43)	702 (46)	1.17 (1.01–1.36)	1.21 (1.03–1.44)	1.10 (0.80–1.52)	1.18 (0.81–1.70)	1.08 (0.68–1.72)
Use pre- or no-surgery[3]							
None	835 (57)	821 (54)	1.0	1.0	1.0	1.0	1.0
>0–10 years	193 (13)	200 (13)	1.13 (0.90–1.41)	1.26 (0.98–1.63)	0.79 (0.47–1.33)	1.05 (0.59–1.85)	1.08 (0.52–2.27)
>10–25 years	214 (15)	213 (14)	1.08 (0.87–1.34)	1.03 (0.80–1.32)	1.34 (0.86–2.08)	1.14 (0.67–1.94)	0.96 (0.48–1.90)
>25 years	228 (16)	289 (19)	1.29 (1.04–1.58)	1.34 (1.06–1.68)	1.21 (0.75–1.97)	1.31 (0.80–2.16)	1.18 (0.63–2.22)
p-Value (trend)			*0.021*	*0.022*	*0.27*	*0.28*	*0.69*
Use post-surgery							
None	1,294 (88)	1,340 (88)	1.0	1.0	1.0	1.0	1.0
>0–10 years	49 (3)	50 (3)	1.08 (0.71–1.62)	1.07 (0.67–1.69)	1.39 (0.60–3.19)	0.97 (0.34–2.77)	0.64 (0.15–2.81)
>10–25 years	81 (6)	87 (6)	1.14 (0.82–1.57)	1.03 (0.72–1.48)	2.04 (1.09–3.79)	1.03 (0.45–2.32)	0.44 (0.11–1.88)
>25 years	46 (3)	46 (3)	1.00 (0.64–1.51)	1.09 (0.69–1.71)	0.91 (0.27–3.05)	0.79 (0.23–2.64)	0.43 (0.06–3.22)
p-Value (trend)			*0.61*	*0.60*	*0.12*	*0.81*	*0.16*
Ever[3] vs. never use stratified by age at diagnosis/recruitment							
<50 years	143 (23)	137 (20)	1.16 (0.86–1.57)	1.53 (1.06–2.19)	1.42 (0.89–2.25)	0.66 (0.28–1.55)	0.98 (0.41–2.29)
50–59 years	213 (33)	237 (34)	1.22 (0.93–1.59)	1.20 (0.89–1.62)	0.76 (0.46–1.26)	1.41 (0.78–2.54)	1.67 (0.88–3.15)
60–69 years	191 (30)	207 (29)	0.93 (0.70–1.23)	0.95 (0.70–1.29)	0.83 (0.49–1.40)	1.31 (0.62–2.75)	0.87 (0.40–1.85)
≥70 years	88 (14)	121 (17)	1.61 (1.10–2.36)	1.66 (1.08–2.56)	0.91 (0.42–1.97)	1.32 (0.50–3.49)	1.41 (0.58–3.35)

[1]Numbers may not sum to total because of missing data.–[2]Adjusted for age (except age-stratified analysis), education, parity and oral contraceptive pill use.–[3]Analysis restricted to use while the genital tract was unobstructed (i.e., prior to hysterectomy).

portion of use in younger women would have been after that date. Significantly elevated risks of ovarian cancer overall and for the serous subtype were seen in women who were 70 years of age or older and also among those who were less than 50 for the serous subtype only. A modest increase in risk was also observed in the 50–59 year group (nonsignificant) however no association was observed in the 60–69 year age group. Similar results were observed when invasive tumours were examined separately (the number of LMP tumours was too small to evaluate the effects by age).

Table III shows no significant association was observed between PID and risk of all subtypes of ovarian cancer combined (OR = 1.15, 95% CI: 0.85–1.57), or for the different histological subtypes. When we examined the association relative to the time elapsed since diagnosis of PID, no association with ovarian cancer risk was observed (data not shown).

A reported history of genital herpes was not associated with risk of all subtypes of ovarian cancer combined (OR = 1.17, 95% CI: 0.73–1.87). However, a significant positive association was seen with risk of serous tumours (OR = 1.65, 95% CI: 1.01–2.69; Table III), with similar nonsignificant increases observed for both invasive (OR = 1.65, 95% CI: 0.98–2.78) and LMP serous tumours (OR = 1.76, 95% CI: 0.71–4.34). For serous tumours, similar increased risks were seen for both more recent (less than 20 years) and long-term (over 20 years) infection (data not shown).

Neither HPV infection, based on self-reported history of abnormal pap smears and/or genital warts, nor a history of mumps after the age of puberty were associated with risk of ovarian cancer overall (Table III). There was also no association with mumps when we considered infection at any age (OR = 0.95, 95% CI: 0.81–1.12). There was however a suggestion that HPV infection was associated with a slightly increased risk of the endometrioid subtype (OR = 1.58, 95% CI: 1.03–2.44). Analyses considering time since the condition was first reported did not alter these results.

We found no significant association between a reported history of endometriosis and ovarian cancer risk overall (OR = 1.31, 95% CI: 0.97–1.78). However statistically significant increased risks were seen for the endometrioid and clear cell subtypes (OR = 1.85, CI: 1.02–3.38 and OR = 2.66, CI: 1.31–5.44, respectively). Because endometriosis may go undiagnosed, we also considered a reported history of potential symptoms of endometriosis (long or painful periods) however neither was associated with ovarian cancer risk (Table III). Similar results were noted when the analysis was restricted to women who had not used hormonal contraceptives. As with other medical conditions, risk estimates did not vary with time elapsed since endometriosis was first reported.

For comparison with inflammation believed to occur in close proximity to the ovaries, medical conditions associated with inflammation at other body sites were also examined (including gall stones, inflammatory bowel disease, diverticulitis, oesophagitis, gastritis and pancreatitis). None of these conditions was associated with ovarian cancer risk (data not shown).

To assess whether regular use of anti-inflammatory medications was inversely associated with ovarian cancer risk, use of aspirin and NSAIDs in the 5 years prior to study recruitment was examined. Any use of aspirin was not associated with ovarian cancer risk for all subtypes combined (OR for any *vs.* no use = 1.06, 95% CI: 0.92–1.23; Table IV) or for any of the individual subtypes. Ever use of NSAIDs in the last 5 years also had no effect on risk of all subtypes of ovarian cancer (OR = 0.88, 95% CI: 0.76–1.02). However, risk of mucinous tumours was inversely associated with any use of NSAIDs (OR = 0.69, 95% CI: 0.50–0.94) and a further decrease in risk was observed with more frequent use (p-value trend = 0.01). Separate analyses of invasive (n = 44) and LMP (n = 147) mucinous tumours demonstrated that the observed inverse association was driven entirely by LMP tumours (OR for any *vs.* no use = 0.59, 95% CI: 0.41–0.84, compared to

TABLE III – ASSOCIATION BETWEEN SELF-REPORTED MEDICAL CONDITIONS POTENTIALLY ASSOCIATED WITH INFLAMMATION OF THE OVARIES AND RISK OF EPITHELIAL OVARIAN CANCER

	Controls[1] *N* (%)	All cases[1] *N* (%)	All cases (*N* = 1,576) OR[2] (95% CI)	Serous (*N* = 994) OR[2] (95% CI)	Mucinous (*N* = 191) OR[2] (95% CI)	Endometrioid (*N* = 141) OR[2] (95% CI)	Clear cell (*N* = 88) OR[2] (95% CI)
PID							
Never	1,406 (94)	1,460 (93)	1.0	1.0	1.0	1.0	1.0
Ever	84 (6)	103 (7)	1.15 (0.85–1.57)	0.96 (0.66–1.38)	1.46 (0.82–2.60)	1.29 (0.66–2.52)	0.87 (0.30–2.49)
Genital herpes							
Never	1,420 (98)	1,425 (97)	1.0	1.0	1.0	1.0	1.0
Ever	35 (2)	42 (3)	1.17 (0.73–1.87)	1.65 (1.01–2.69)	0.40 (0.09–1.71)	0.32 (0.04–2.37)	0.74 (0.10–5.63)
HPV infection							
Never	1,148 (78)	1,197 (81)	1.0	1.0	1.0	1.0	1.0
Ever	317 (22)	273 (19)	0.94 (0.78–1.15)	0.92 (0.74–1.15)	0.98 (0.66–1.45)	1.58 (1.03–2.44)	0.72 (0.36–1.47)
Mumps							
Never	496 (76)	508 (75)	1.0	1.0	1.0	1.0	1.0
Ever (postpubertal)	160 (24)	164 (25)	0.96 (0.73–1.25)	1.06 (0.79–1.42)	0.78 (0.40–1.49)	0.97 (0.50–1.87)	0.81 (0.35–1.92)
Endometriosis[3]							
Never	1,413 (94)	1,431 (92)	1.0	1.0	1.0	1.0	1.0
Ever	87 (6)	124 (8)	1.31 (0.97–1.78)	1.14 (0.80–1.62)	0.89 (0.46–1.75)	1.85 (1.02–3.38)	2.66 (1.31–5.44)
Long periods[3] (>7 days)							
Never/rarely	1,174 (82)	1,173 (82)	1.0	1.0	1.0	1.0	1.0
Often	188 (13)	192 (14)	1.05 (0.83–1.31)	1.05 (0.81–1.36)	0.70 (0.40–1.22)	1.23 (0.71–2.12)	1.26 (0.62–2.53)
Always	75 (5)	62 (4)	0.79 (0.55–1.13)	0.82 (0.55–1.23)	0.78 (0.34–1.78)	0.72 (0.27–1.85)	0.83 (0.24–2.83)
Painful periods[3]							
Never/rarely	760 (52)	711 (49)	1.0	1.0	1.0	1.0	1.0
Sometimes	290 (20)	301 (20)	1.04 (0.85–1.27)	1.04 (0.83–1.31)	0.95 (0.61–1.47)	1.07 (0.65–1.75)	1.13 (0.59–2.15)
Often	404 (28)	452 (31)	1.17 (0.98–1.40)	1.17 (0.96–1.43)	1.12 (0.77–1.64)	1.12 (0.72–1.73)	1.14 (0.65–2.00)

[1]Numbers may not sum to total because of missing data.–[2]Adjusted for age, education, parity and oral contraceptive pill use.–[3]Additionally adjusted for body mass index one year prior to diagnosis.

TABLE IV – ASSOCIATION BETWEEN ANTI-INFLAMMATORY MEDICATION USE IN THE PAST 5 YEARS AND RISK OF EPITHELIAL OVARIAN CANCER

	Controls[1] *N* (%)	All cases[1] *N* (%)	All cases (*N* = 1,576) OR[2] (95% CI)	Serous (*N* = 994) OR[2] (95% CI)	Mucinous (*N* = 191) OR[2] (95% CI)	Endometrioid (*N* = 141) OR[2] (95% CI)	Clear cell (*N* = 88) OR[2] (95% CI)
Aspirin							
Never	772 (51)	783 (50)	1.0	1.0	1.0	1.0	1.0
Ever	730 (49)	781 (49)	1.06 (0.92–1.23)	1.06 (0.90–1.25)	0.99 (0.72–1.35)	0.92 (0.64–1.32)	0.92 (0.58–1.45)
≤1/week	612 (41)	650 (41)	1.06 (0.91–1.23)	1.05 (0.88–1.25)	0.98 (0.71–1.36)	0.98 (0.68–1.43)	0.95 (0.59–1.54)
≥2/week	118 (8)	131 (8)	1.06 (0.80–1.41)	1.11 (0.81–1.51)	1.02 (0.52–2.03)	0.56 (0.23–1.34)	0.75 (0.30–1.89)
p-Value (trend)			*0.5*	*0.4*	*0.99*	*0.4*	*0.6*
NSAIDs							
Never	625 (42)	723 (46)	1.0	1.0	1.0	1.0	1.0
Ever	878 (58)	836 (54)	0.88 (0.76–1.02)	0.93 (0.78–1.10)	0.69 (0.50–0.94)	0.76 (0.53–1.09)	0.92 (0.58–1.45)
≤1/week	653 (43)	625 (40)	0.90 (0.76–1.05)	0.94 (0.78–1.12)	0.73 (0.53–1.02)	0.73 (0.50–1.09)	0.97 (0.59–1.60)
≥2/week	225 (15)	211 (14)	0.83 (0.66–1.04)	0.90 (0.70–1.16)	0.51 (0.28–0.93)	0.84 (0.49–1.44)	0.79 (0.39–1.58)
p-Value (trend)			*0.1*	*0.3*	*0.01*	*0.3*	*0.6*

[1]Numbers may not sum to total because of missing data.–[2]Adjusted for age, education, parity and oral contraceptive pill use.

1.17, 95% CI 0.62–2.21 for invasive mucinous tumours). There was also a dose-response relationship for LMP mucinous tumours (OR for 2 or more pills per week *vs.* no use = 0.46, 95% CI: 0.23–0.91, *p*-value trend = 0.01).

Discussion

The hypothesis that chronic inflammation may lead to the development of epithelial ovarian cancer was first proposed to explain how certain factors, such as talc use in the perineal region, may be linked to increased risk of developing ovarian cancer.[1] Testing the inflammation hypothesis in a case–control study, Ness *et al.* found that proinflammatory factors, such as perineal talc use and endometriosis increased ovarian cancer risk, but others such as PID did not significantly increase ovarian cancer risk (separate analyses of individual histological subtypes of ovarian cancer were not presented).[8] Consistent with this hypothesis, McSorley *et al.*[9] recently reported a trend of increasing ovarian cancer risk with increasing levels of CRP, a marker of inflammation. However, given the lack of specificity of CRP and its association with prevalent chronic conditions, such as ischaemic heart disease,[12] it is difficult to rule out confounding as an alternate explanation for these results.[9] Until the present study, no other epidemiological studies appear to have tested the hypothesis that ovarian inflammation is associated with ovarian cancer risk. In the current study, a significantly elevated risk of ovarian cancer overall and of the serous subtype associated with perineal talc use was identified. A nonsignificant increase in risk was also seen for endometrioid tumours. Other factors that could potentially cause ovarian inflammation (such as PID, HPV infection, mumps and endometriosis) were not associated with ovarian cancer risk overall, however there was some evidence of a positive association with some of these factors in the subtype specific analyses. These results in combination with previous studies suggest that chronic inflammation is unlikely to play a major role in the development of ovarian cancer.

Focusing on talc use, we found that any use of perineal talc was associated with a small but significantly increased risk of ovarian cancer overall and specifically amongst the invasive and LMP serous tumours although no clear dose-response with increasing duration of use was identified. This finding is consistent with results of previous studies.[4,6,7,10,13,14]

As expected, ovarian cancer risk was only related to talc use in women with no surgical closure of the fallopian tubes or those who had used talc presurgery, with no association seen for talc use after tubal sterilisation or hysterectomy. Similar observations were made in previous case–control studies of ovarian cancer (all subtypes) with elevated risks observed in women who had not had a tubal ligation[4,14] or those who had used talc presurgery.[13] These former studies together with the current findings support the hypothesis that talc particles are transported to the ovaries *via* unobstructed fallopian tubes. In contrast, the Nurses' Health study found no increase in risk among women who were perineal talc users but had never had a tubal ligation.[7]

While it has been demonstrated experimentally that talc particles can reach the ovaries in humans and rodents as the result of talc use in the pelvic region,[15–17] ovarian talc particle burden in normal human ovaries is not correlated with reported exposure levels.[17] This suggests that use of only a small amount of talc may be required for some talc to reach the ovaries and increase risk of cancer.

It has been hypothesised that talc is linked to ovarian cancer development through inflammation, however evidence linking an inflammatory response with talc contamination of the ovaries is lacking. Talc-induced inflammation is unlikely to be in the formation of granulomas as these are rarely observed in human ovaries.[18,19] Other likely manifestations of talc-induced inflammation include reduced fibrinolysis, activation of neutrophils and macrophages and increased production of cytokines and growth factors, and these have been suggested to occur in the peritoneum in response to contamination by surgical glove powder.[20] Rigorous investigation of the precise biological response of the ovarian surface epithelium to perineal talc use is needed.

We also sought to determine whether possible contamination of talc with asbestos fibres, which are known to cause inflammation of epithelial tissues, could explain the observed link between perineal talc use and serous ovarian cancer. Voluntary guidelines to prevent asbestos contamination of cosmetic talc were introduced in 1976 and consequently earlier formulations were more likely to contain asbestos fibres.[10,11] Increased risk of serous ovarian cancer was not restricted to perineal talc use in the oldest age groups, who were more likely to have been exposed to asbestos-contaminated talc, but was also observed in the youngest (less than 50 years) and the 50–59 year old age group. Other studies have also reported no increase in risk of all subtypes of ovarian cancer associated with talc use before 1970[13] or before 1975.[14] These findings contrast with 2 other reports of increased risk of serous[7] and all subtypes of epithelial ovarian cancer[10] associated with earlier use of talc.

If inflammation plays a role in the aetiology of ovarian cancer then it would be expected that PID would be associated with increased risk of ovarian cancer. PID was not associated with elevated risk of ovarian tumours in our data, confirming several previous reports of no association with PID in studies of all subtypes of ovarian cancer.[8,21,22] To date there has been only one report of a significant positive association between PID and ovarian cancer.[23] Genital herpes infection was associated with a nonsignificant increased risk of invasive serous cancer in our data, although this observation was based on a small number of exposed cases ($n = 27$). One previous study found no association between genital herpes and ovarian cancer risk (the number of exposed cases was not reported).[8] Latent infection by herpes virus is established

in the nerve root ganglia and it is associated with a variety of initial and recurrent symptoms such as genital ulceration.[24] It is biologically plausible that inflammation associated with genital herpes infection could increase risk of ovarian cancer as Herpes simplex virus type 2 has been detected in the upper genital tract of women with acute PID[25,26] and acute salpingitis.[27] Further studies are needed to confirm this association.

HPV infection (based on reports of abnormal pap smears and/or genital warts) showed no association with ovarian cancer risk, except for the endometrioid subtype. We hypothesised that HPV infection could potentially cause ovarian inflammation as HPV DNA has been identified in the ovaries of patients with primary ovarian squamous intraepithelial neoplasia[28,29] and in the upper genital tract of patients with cervical squamous carcinoma.[30] In addition, high-risk HPV DNA has been reported in 10% of ovarian epithelial carcinomas.[31] Abnormal pap smears and genital warts are generally associated with HPV genotypes classified as high-risk and low-risk, respectively, in regards to their association with carcinogenic transformation.[32] However, separate analyses also showed no association with ovarian cancer risk.

Mumps infection (either after puberty or at any age) was not associated with ovarian cancer risk. It has been estimated that some 5% of postpubertal mumps cases are associated with clinically apparent oophoritis, which in severe cases could result in infertility caused by nonfunctional ovarian tissue.[33] We were unable to identify these particular cases in the current analysis and therefore further study is needed to examine the association between mumps oophoritis and ovarian cancer.

While endometriosis is a condition associated with localised inflammation, it is also related to changes in hormone levels (increased oestrogen unopposed by progesterone) at the site of endometriotic implants.[34] Despite this, endometriosis or potential symptoms of endometriosis (long or painful periods) were not associated with ovarian cancer risk overall, but there was an increased risk of endometrioid and clear cell subtypes among women who reported a history of endometriosis. This result was anticipated because current epidemiological evidence suggests that endometriosis is most strongly associated with the endometrioid and clear cell subtypes of ovarian cancer.[35,36]

Finally, if inflammation did promote epithelial ovarian cancer development, then it may be reasonably expected that regular use of anti-inflammatory medications would reduce risk. However, no overall association with ovarian cancer risk was observed in the current study. This supports results from 2 recent meta-analyses, which have also not shown that regular use of anti-inflammatory medications (aspirin or other NSAIDs) reduces ovarian cancer risk.[37,38] Of interest however was the apparent inverse association between NSAID use and the mucinous subtype, which was entirely driven by the LMP group. We know from other epidemiological studies that the aetiology of mucinous tumours differs in a number of ways from the other subtypes of ovarian cancer, so NSAID use may be another factor to add to this list. However, this result awaits confirmation by others.

Strengths of our study included its large size (1,576 women with ovarian cancer and 1,509 population-based controls) and Australia-wide coverage. A limitation was the low response rate for controls (47%), which could have resulted in selection bias and possibly led to an over-representation of healthy subjects among the controls. Indeed our hysterectomy rate among controls was ~5% lower than expected, but as there are no obvious links between hysterectomy and inflammation that we have not considered, we do not believe that these small differences would have affected the present results. A healthy control bias would most likely influence the analyses of medical conditions, specifically sexually transmitted infections (STIs). For example, if participating controls were less likely to have had an STI this could bias risk estimates for STIs upwards. While we saw a positive association between herpes infection and ovarian cancer risk, there was no association with other STIs suggesting that our ORs are not systematically biased. Overall, a small number of participants reported STIs and it is possible that STIs were underreported because of possible asymptomatic infection or because of the negative connotations associated with having an STI. It is also possible that controls would be more likely to underreport STIs than cases therefore potentially biasing the risk estimates upwards. Another general limitation was that analyses of medical conditions were based entirely on self-reported medical history and as a result the accuracy of these reports could not be confirmed, although self-reports of these miscellaneous conditions are unlikely to be influenced greatly by case/control status.

In summary, most factors that could potentially cause ovarian inflammation (such as PID, HPV infection, and postpubertal mumps) were not associated with a significant elevation in ovarian cancer risk in our study. In addition, the expected corollary, an inverse association with regular use of anti-inflammatory medications, was not observed. While some subtype-specific associations were observed, these were not strong and showed no coherent pattern of association within or across subtypes, aside from the well-recognised increase in risk of endometrioid and clear cell cancers among women with endometriosis. The elevation in ovarian cancer risk associated with use of talc in the perineal region that we and others have observed has been regarded as the main evidence supporting an inflammatory mechanism in the development of epithelial ovarian cancer. However, experimental evidence that perineal talc use elicits an inflammatory response in the ovaries is lacking and overall we conclude that chronic inflammation does not play a major role in the development of ovarian cancer.

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Exhibit 44

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Original Contribution

Ovarian Cancer Risk Factors in African-American and White Women

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Ovarian cancer is the most lethal gynecologic malignancy in both African-American and white women. Although prevalences of many ovarian cancer risk factors differ markedly between African Americans and whites, there has been little research on how the relative contributions of risk factors may vary between racial/ethnic groups. Using data from a North Carolina case-control study (1999–2008), the authors conducted unconditional logistic regression analyses to calculate odds ratios and 95% confidence intervals for ovarian cancer risk factors in African-American (143 cases, 189 controls) and white (943 cases, 868 controls) women and to test for interactions by race/ethnicity. They also calculated attributable fractions within each racial/ethnic group for the modifiable factors of pregnancy, oral contraceptive use, tubal ligation, and body mass index. Many risk factors showed similar relations across racial/ethnic groups, but tubal ligation and family history of breast or ovarian cancer showed stronger associations among African Americans. Younger age at menarche was associated with risk only in white women. Attributable fractions associated with tubal ligation, oral contraceptive use, and obesity were markedly higher for African Americans. The relative importance of ovarian cancer risk factors may differ for African-American women, but conclusions were limited by the small sample. There is a clear need for further research on etiologic factors for ovarian cancer in African-American women.

African Americans; case-control studies; ovarian neoplasms

Abbreviations: BMI, body mass index; CI, confidence interval.

Ovarian cancer is the eighth most common cancer among both white and African-American women and the fifth most common cause of cancer death in the United States (1, 2). African-American women have lower incidence rates than white women (10.1 cases/100,000 women vs. 14.1 cases/100,000 women) but poorer 5-year survival (1). Despite the importance of ovarian cancer as a major cause of morbidity and mortality, there has been very little research on ovarian cancer among African Americans. Only 2 published papers have focused on risk factors for ovarian cancer among African Americans: 1 on a case-control study with 84 cases (3) and 1 on a multicenter analysis of 7 case-control studies involving 110 cases (4). Both of these reports had findings that were consistent with the major reproductive risk factors identified in white women, including inverse associations with parity and oral contraceptive use (3, 4), but some racial/ethnic differences were noted, including the absence of a protective effect for breastfeeding and no increased risk associated with a family history of ovarian cancer among African Americans (3).

As has been reported by John et al. (4), Ness et al. (3), and other authors (5–12), the prevalence of many factors associated with risk of ovarian cancer varies markedly between African Americans and whites. African-American women tend to have a greater number of pregnancies (5, 7), a higher prevalence of tubal ligation (6), a lower prevalence of endometriosis (9), and less use of menopausal hormones (5, 10), all of which would be associated with a lower incidence of ovarian cancer. They also tend to have an earlier age at menarche (11), are more likely to be obese (12), and are less likely to breastfeed (8), which could contribute to higher risk of ovarian cancer. Because most epidemiologic

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studies of ovarian cancer have enrolled very few African-American women, there is little information on the relative importance of these risk factors among African-American women as compared with white women and the extent to which differences in the prevalence of established risk factors can explain the lower incidence of ovarian cancer among African Americans.

In this paper, we use data from the North Carolina Ovarian Cancer Study to compare risk factors for ovarian cancer among African-American and white women. We also calculate population attributable fractions for risk factors that are both modifiable and show considerable racial/ethnic differences in prevalence to evaluate the relative proportions of cases in African-American and white women that are associated with these factors.

MATERIALS AND METHODS

The North Carolina Ovarian Cancer Study was a population-based, case-control study of epithelial ovarian cancer that was conducted in a 48-county region of North Carolina between 1999 and 2008. Newly diagnosed cases of epithelial ovarian cancer were identified through the North Carolina Central Cancer Registry using a rapid case ascertainment system. Pathology reports for eligible cases were sent to the study office at Duke University Medical Center, and consent to contact the women was requested from the treating physicians. Eligible cases were aged 20–74 years at diagnosis, had no prior history of ovarian cancer, resided in the study area, and were cognitively able to give consent and to complete an interview in English. All cases underwent standardized histopathologic review by the study pathologist for confirmation of the diagnosis. Control women were frequency-matched by age and race/ethnicity to the cases and were recruited from the same geographic region using list-assisted random digit dialing. The eligibility criteria were the same as those for the cases; in addition, the controls could not have had a bilateral oophorectomy.

The response rate among the cases was 66.5%, with nonparticipation being due to death (4.0%), debilitating illness (2.6%), physician refusal (4.7%), patient refusal (11.5%), or an inability to locate the patient (10.7%). Among potential controls, screening for eligibility could not be completed for 14% of phone numbers. Seventy-three percent of potential controls who passed eligibility screening agreed to be sent information about the study, and 60.1% of those consented to be in the study. Nonparticipation was due to refusal (27.4%) or an inability to contact the person (8.8%). Response rates were lower for African Americans than for whites (56.6% and 68.3%, respectively, for cases and 49.7% and 63.7%, respectively, for controls).

A total of 1,114 cases were enrolled, of whom 943 (84.6%) were white, 143 (12.8%) were African-American, and 28 (2.5%) were of other races/ethnicities. Among the 1,086 controls, 868 (79.9%) were white, 189 (17.4%) were African-American, and 29 (2.7%) were of other races/ethnicities. The analyses in this report were limited to women whose self-reported race/ethnicity was either white or African-American. The study protocol was approved by the Duke University Medical Center Institutional Review Board and by the human subjects committees at the North Carolina Central Cancer Registry and each hospital where cases were identified.

Table 1. Clinical and Histologic Characteristics of Epithelial Ovarian Cancer Cases in African-American and White Women, North Carolina Ovarian Cancer Study, 1999–2008

	Whites		African Americans		*P* Value[a]
	No.	%	No.	%	
All cases	($n = 943$)		($n = 143$)		
Invasive tumor	746	79.4	111	77.6	0.64
Low-malignant-potential tumor	194	20.6	32	22.4	
Missing data	3		0		
Invasive cases only	($n = 746$)		($n = 111$)		
Histologic type					
Serous	419	56.2	67	60.4	0.05
Clear-cell	82	11.0	2	1.8	
Endometrioid	116	15.5	19	17.1	
Mucinous	39	5.2	6	5.4	
Other	90	12.1	17	15.3	
Stage					
I or II	245	33.1	25	22.7	0.04
III or IV	496	66.9	85	77.3	
Missing data	5		1		
Grade					
Well-differentiated	93	12.9	18	16.8	0.12
Moderately differentiated	197	27.2	36	33.6	
Poorly differentiated or undifferentiated	433	59.9	53	49.5	
Missing data	23		4		

[a] *P* values were derived from chi-squared analyses.

Nurse-interviewers conducted in-person visits at which they obtained written informed consent, administered a 90-minute questionnaire, drew a blood sample, and performed anthropometric measurements (height, weight, and waist and hip circumferences). Information obtained with the questionnaire included family history of cancer; menstrual characteristics such as age at menarche and cycle length; reproductive history, including age at each pregnancy, pregnancy duration and outcome, and duration of breastfeeding; type, timing, and duration of hormone and contraceptive use; and lifestyle characteristics such as smoking history, alcohol consumption during the 5 years before interview, and physical activity. A life-events calendar, which marked milestones such as marriages and births, was used to aid recall of reproductive history and hormone use. Pictures of oral contraceptives, menopausal hormones, and certain other medications were also used to assist with recall.

Statistical analysis

Chi-squared analyses were used to compare clinical and histologic characteristics of cases between African Americans

Table 2. Characteristics of Invasive Epithelial Ovarian Cancer Cases and Controls, by Race/Ethnicity, North Carolina Ovarian Cancer Study, 1999–2008

	Whites						African Americans					
	Cases (n = 746)		Controls (n = 868)		OR[a]	95% CI	Cases (n = 111)		Controls (n = 189)		OR[a]	95% CI
	No.	%	No.	%			No.	%	No.	%		
Age, years												
20–39	38	5.1	81	9.3			11	9.9	22	11.6		
40–49	136	18.2	170	19.6			23	20.7	46	24.3		
50–59	239	32.0	261	30.1			37	33.3	67	35.4		
60–69	232	31.1	240	27.6			31	27.9	40	21.2		
70–74	101	13.5	116	13.4			9	8.1	14	7.4		
Age at menarche, years												
<12	181	24.4	157	18.2	1.00	Referent	28	25.5	53	28.0	1.00	Referent
≥12	562	75.6	708	81.8	0.67	0.53, 0.86	82	74.5	136	72.0	1.08	0.63, 1.85
Missing data	3		3				1		0			
No. of pregnancies								11				
0	120	16.1	87	10.0	1.00	Referent	14	12.6	11	5.8	1.00	Referent
1–2	319	42.8	348	40.1	0.62	0.45, 0.85	31	27.9	71	37.6	0.34	0.14, 0.82
≥3	307	41.2	433	49.9	0.45	0.33, 0.62	66	59.5	107	56.6	0.44	0.19, 1.05
P-trend					<0.0001		0		0		0.25	
Age at first pregnancy, years												
<20	173	27.6	202	25.9	1.00	Referent	56	58.3	94	52.8	1.00	Referent
20–24	276	44.1	333	42.7	0.93	0.72, 1.21	30	31.3	52	29.2	0.98	0.56, 1.72
25–29	137	21.9	151	19.4	1.09	0.80, 1.49	8	8.3	19	10.7	0.73	0.30, 1.79
30–34	31	5.0	79	10.1	0.50	0.31, 0.79	1	1.0	10	5.6	0.18	0.02, 1.45
≥35	9	1.4	14	1.8	0.77	0.33, 1.84	1	1.0	3	1.7	0.65	0.07, 6.45
Missing data	120		89				15		11			
P-trend					0.0004						0.15	
Age at last pregnancy, years												
<20	19	3.0	18	2.3	1.00	Referent	11	11.7	13	7.3	1.00	Referent
20–24	134	21.4	147	18.9	0.79	0.39, 1.57	24	25.5	35	19.8	0.82	0.31, 2.14
25–29	233	37.3	258	33.1	0.76	0.38, 1.49	25	26.6	51	28.8	0.57	0.22, 1.45
30–34	161	25.8	230	29.5	0.60	0.30, 1.18	22	23.4	48	27.1	0.54	0.21, 1.40
≥35	78	12.5	126	16.2	0.53	0.26, 1.08	12	12.8	30	16.9	0.43	0.15, 1.23
Missing data	121		89				17		12			
P-trend					<0.0001						0.04	
Ever breastfeeding												
No	521	69.8	542	62.4	1.00	Referent	75	67.6	135	71.4	1.00	Referent
Yes	225	30.2	326	37.6	0.73	0.59, 0.90	36	32.4	54	28.6	1.16	0.69, 1.93
Missing data	0		0				0		0			
Tubal ligation												
No	559	75.0	579	66.8	1.00	Referent	77	69.4	93	49.2	1.00	Referent
Yes	186	25.0	288	33.2	0.68	0.54, 0.84	34	30.6	96	50.8	0.43	0.26, 0.71
Missing data	1		1				0		0			
Duration of oral contraceptive use, years												
Never use	244	34.5	239	28.3	1.00	Referent	47	43.9	58	32.2	1.00	Referent
<1	99	14.0	92	10.9	1.09	0.77, 1.52	15	14.0	14	7.8	1.36	0.59, 3.14
1–<5	166	23.4	228	27.0	0.75	0.57, 0.99	24	22.4	57	31.7	0.54	0.28, 1.04
≥5	199	28.1	285	33.8	0.73	0.55, 0.96	21	19.6	51	28.3	0.53	0.27, 1.03
Missing data	38		24				4		9			

Table continues

Table 2. Continued

	Whites						African Americans					
	Cases (n = 746)		Controls (n = 868)		OR[a]	95% CI	Cases (n = 111)		Controls (n = 189)		OR[a]	95% CI
	No.	%	No.	%			No.	%	No.	%		
Use of menopausal hormones												
No	276	37.0	456	52.6	1.00	Referent	75	68.8	148	78.3	1.00	Referent
Yes	470	63.0	411	47.4	1.85	1.50, 2.28	34	31.2	41	21.7	1.54	0.90, 2.66
Missing data	0		1				2		0			
Hysterectomy												
No	537	72.2	667	76.9	1.00	Referent	82	73.9	145	76.7	1.00	Referent
Yes	207	27.8	200	23.1	1.22	0.97, 1.54	29	26.1	44	23.3	1.07	0.61, 1.87
Missing data	2		1				0		0			
History of infertility												
No	651	87.3	783	90.2	1.00	Referent	102	91.9	175	92.6	1.00	Referent
Yes	95	12.7	85	9.8	1.38	1.01, 1.89	9	8.1	14	7.4	1.13	0.47, 2.73
Missing data	0		0				0		0			
History of endometriosis												
No	650	87.7	793	92.3	1.00	Referent	109	98.2	184	98.4	1.00	Referent
Yes	91	12.3	66	7.7	1.76	1.26, 2.46	2	1.8	3	1.6	1.16	0.19, 7.08
Missing data	5		9				0		2			
First-degree family history of breast or ovarian cancer												
No	582	78.1	720	83.1	1.00	Referent	69	62.2	159	84.1	1.00	Referent
Yes	163	21.9	146	16.9	1.33	1.04, 1.71	42	37.8	30	15.9	3.15	1.82, 5.45
Missing data	1		2				0		0			
Talc use												
No	328	59.6	325	61.0	1.00	Referent	45	54.2	75	56.0	1.00	Referent
Yes	222	40.4	208	39.0	1.04	0.82, 1.33	38	45.8	59	44.0	1.19	0.68, 2.09
Missing data	196		335				28		55			
Body mass index[b] 1 year before diagnosis or interview												
<25	312	43.3	369	43.7	1.00	Referent	17	15.9	31	17.1	1.00	Referent
25–<30	212	29.4	256	30.3	0.96	0.76, 1.22	26	24.3	58	32.0	0.84	0.39, 1.78
30–<35	114	15.8	124	14.7	1.08	0.80, 1.45	22	20.6	43	23.8	0.94	0.43, 2.07
≥35	83	11.5	95	11.3	1.04	0.75, 1.45	42	39.3	49	27.1	1.62	0.79, 3.35
Missing data	25		24				4		8			
Height, m												
<1.6	195	26.2	242	27.9	1.00	Referent	25	22.7	57	30.2	1.00	Referent
1.6–<1.7	430	57.8	483	55.8	1.13	0.90, 1.42	64	58.2	102	54.0	1.48	0.84, 2.62
≥1.7	119	16.0	141	16.3	1.11	0.81, 1.51	21	19.1	30	15.9	1.74	0.83, 3.65
Missing data	2		2				1		0			

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] Adjusted for age.
[b] Weight (kg)/height $(m)^2$.

and whites. Unconditional logistic regression analyses were used to calculate age-adjusted and multivariable-adjusted odds ratios and 95% confidence intervals separately for each racial/ethnic group. Variables included in the race/ethnicity-specific multivariable models were age, age at menarche, number of pregnancies, duration of oral contraceptive use, history of tubal ligation, family history of breast and ovarian cancer, and body mass index (BMI; weight (kg)/height $(m)^2$). The variables included in multivariable models were selected a priori and included the most well-established risk

factors for ovarian cancer as well as BMI, because of the pronounced racial/ethnic differences in the prevalence of obesity. We also conducted multivariable analyses limited to parous women that included all of the above variables plus breastfeeding. Finally, to test for interactions, we fitted models for women of both racial/ethnic groups combined which included a term for race/ethnicity and product terms for race/ethnicity × age at menarche, race/ethnicity × breastfeeding, and race/ethnicity × family history of breast or ovarian cancer.

Population attributable fractions were calculated using the method described by Bruzzi et al. (13) for the potentially modifiable factors tubal ligation (yes vs. no), oral contraceptive use (≥1 year vs. <1 year), history of pregnancy (ever vs. never), and BMI (<30 vs. ≥30). For these analyses, the reference categories were assigned to the lower risk category (i.e., having had a tubal ligation, oral contraceptive use for ≥1 year, ever being pregnant, and BMI <30) so the attributable fraction could be interpreted as the proportion of cases that theoretically could be eliminated if all women in the population were shifted to the low risk category.

RESULTS

The tumor characteristics of the ovarian cancer cases are presented in Table 1 by race/ethnicity. The proportions of cases that were invasive were similar for African Americans and whites (78% and 79%, respectively). Because low-malignant-potential ovarian cancer may be etiologically distinct from invasive cancer (14, 15), we focused the remainder of our analyses on invasive disease. Among invasive cases, the most important histologic differences were that tumors in African Americans were less likely to be clear-cell and more likely to be of a histologic type other than the 4 primary types (serous, endometrioid, mucinous, and clear-cell). African-American women were more likely to be diagnosed with higher-stage disease and somewhat less likely to have poorly differentiated tumors, although the differences in grade were not statistically significant.

Comparisons of risk factors for ovarian cancer among African-American and white women are presented in Table 2. Because age-matching was based on all cases but this analysis was restricted to invasive cases, who are on average older than low-malignant-potential cases, the age distribution of the controls was slightly younger than that of the cases.

In age-adjusted analyses, many of the major reproductive factors that have been associated with ovarian cancer risk among white women were similarly related to risk among African-American women. Women who were parous, had a later age at last pregnancy, had used oral contraceptives for 1 year or more, or had had a tubal ligation were at reduced risk of invasive ovarian cancer; however, there was not strong evidence of a linear relation with number of pregnancies for African-American women. History of infertility or endometriosis was associated with a significantly increased risk for white women and a modestly but not significantly increased risk for African-American women. Family history of breast or ovarian cancer in a first-degree relative was associated with increased risk in both racial/ethnic groups, with a stronger association among African Americans. Later age at menarche and history of ever breastfeeding were associated with reduced risk in white women, whereas no association was observed among African Americans. Analyses of anthropometric characteristics suggested that taller height and BMI ≥35 may be associated with risk among African-American women but not among white women.

In multivariable models (Table 3), results were generally similar to those observed in the age-adjusted models. The association with age at menarche ≥12 years appeared to differ by race/ethnicity, with an odds ratio of 1.30 (95% confidence interval (CI): 0.67, 2.53) for African Americans rather than the expected inverse association. The strength of the association with family history of breast or ovarian cancer also appeared to differ by race/ethnicity. *P* values for the interaction terms were 0.032 for race/ethnicity × family history and 0.068 for race/ethnicity × age at menarche. In models limited to parous women that included all of the variables in Table 3 plus history of breastfeeding, white women who had breastfed had a nonsignificantly reduced risk (odds ratio = 0.83, 95% CI: 0.65, 1.06), whereas there was no suggestion of a protective effect among African-American women (odds ratio = 1.09, 95% CI: 0.57, 2.07).

In addition to some differences between African Americans and whites in the magnitude of associations with certain risk factors, there were marked racial/ethnic differences in the prevalences of a number of risk factors considered. For example, prevalences in African-American and white controls, respectively, were 29% and 18% for age at menarche less than 12 years, 6% and 10% for nulligravidity, 51% and 33% for tubal ligation, and 51% and 26% for BMI ≥30 (Table 3). We therefore hypothesized that the relative contribution of established risk factors for ovarian cancer could vary considerably between African Americans and whites. To address this, we calculated population attributable fractions for the potentially modifiable risk factors of pregnancy, oral contraceptive use, BMI, and tubal ligation. As Table 4 shows, the attributable fractions for not having a tubal ligation, high BMI, and no oral contraceptive use were considerably higher for African Americans than for whites, reflecting the stronger associations and/or higher prevalence of these factors among African Americans.

DISCUSSION

Our analyses of ovarian cancer risk factors in African-American and white women show similar relations for several characteristics, including inverse associations with parity, oral contraceptive use, and tubal ligation, but there are also suggestions of racial/ethnic differences in either the direction or the magnitude of association for other risk factors. History of breastfeeding and later age at menarche were both associated with reduced risk among whites, whereas these associations were absent among African Americans. Family history of breast or ovarian cancer was associated with increased risk for both African Americans and whites, but the association was considerably stronger for African-American women. We considered the possibility that the

Table 3. Odds Ratios for Invasive Epithelial Ovarian Cancer (Multivariable Logistic Regression Models) in African-American and White Women, North Carolina Ovarian Cancer Study, 1999–2008

	Whites						African Americans					
	Cases		Controls		OR[a]	95% CI	Cases		Controls		OR[a]	95% CI
	No.	%	No.	%			No.	%	No.	%		
Age, years (continuous variable)	715		837		1.01	1.00, 1.02	106		181		1.00	1.00, 1.02
No. of pregnancies												
0	114	15.9	84	10.0	1.00	Referent	14	13.2	11	6.1	1.00	Referent
1–2	306	42.8	332	39.7	0.66	0.47, 0.94	29	27.4	68	37.6	0.28	0.09, 0.86
≥3	295	41.3	421	50.3	0.46	0.32, 0.65	63	59.4	102	56.4	0.52	0.17, 1.62
Age at menarche, years												
<12	172	24.1	151	18.0	1.00	Referent	26	24.5	52	28.7	1.00	Referent
≥12	543	75.9	686	82.0	0.65	0.50, 0.84	80	75.5	129	71.3	1.30	0.67, 2.53
Tubal ligation												
No	535	74.8	561	67.0	1.00	Referent	73	68.9	89	49.2	1.00	Referent
Yes	180	25.2	276	33.0	0.74	0.58, 0.94	33	31.1	92	50.8	0.43	0.24, 0.80
Duration of oral contraceptive use, years												
Never use	233	34.1	225	27.6	1.00	Referent	45	43.3	55	32.0	1.00	Referent
<1	95	13.9	88	10.8	1.18	0.82, 1.69	15	14.4	14	8.1	1.89	0.73, 4.95
1–<5	162	23.7	222	27.2	0.78	0.58, 1.05	23	22.1	55	32.0	0.72	0.34, 1.53
≥5	193	28.3	281	34.4	0.73	0.54, 0.97	21	20.2	48	27.9	0.52	0.24, 1.15
Missing data	32		21				2		9			
Family history of breast or ovarian cancer												
No	559	78.2	697	83.3	1.00	Referent	66	62.3	153	84.5	1.00	Referent
Yes	156	21.8	140	16.7	1.31	1.00, 1.72	40	37.7	28	15.5	2.73	1.45, 5.14
Body mass index[b] 1 year before diagnosis/ interview												
<25	309	43.2	368	44.0	1.00	Referent	17	16.0	31	17.1	1.00	Referent
25–<30	211	29.5	254	30.3	0.92	0.71, 1.18	26	24.5	58	32.0	0.96	0.40, 2.30
30–<35	112	15.7	122	14.6	1.17	0.85, 1.61	22	20.8	43	23.8	1.32	0.53, 3.26
≥35	83	11.6	93	11.1	1.03	0.72, 1.47	41	38.7	49	27.1	1.52	0.65, 3.56

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] Adjusted for all of the variables in the table.
[b] Weight (kg)/height $(m)^2$.

stronger association in African-American women was due to inaccurate reporting; however, the prevalences of a family history of breast or ovarian cancer were very similar among African-American and white controls, which argues against there being differential reporting of family history across racial/ethnic groups.

Although these observed racial/ethnic differences in the magnitude or direction of associations with established ovarian cancer risk factors are intriguing, the limitations of our analyses must be acknowledged. The North Carolina Ovarian Cancer Study included more African-American women than any other study of ovarian cancer, but the relatively small sample made it difficult to ascertain which associations were true associations and which were chance findings.

The modest sample size also precluded us from conducting analyses within subgroups defined by either menopausal status or histologic type. Several reports have suggested that reproductive risk factors and high BMI are more strongly associated with premenopausal disease (16–23). However, with only 38 premenopausal African-American cases in our study population, analyses stratified by menopausal status would not have yielded meaningful results. Similarly, the sample was too small for us to explore differences in risk factors by histologic subtype. The relatively small number of African-American cases also led us to dichotomize some

Table 4. Odds Ratios for Invasive Epithelial Ovarian Cancer and Population Attributable Fractions for Selected Ovarian Cancer Risk Factors in African-American and White Women, North Carolina Ovarian Cancer Study, 1999–2008

	Whites					African Americans				
	No. of Cases	No. of Controls	OR[a]	95% CI	AF	No. of Cases	No. of Controls	OR[a]	95% CI	AF
Tubal ligation										
Yes	168	269	1.00	Referent	0.204	33	91	1.00	Referent	0.341
No	515	547	1.37	1.08, 1.73		71	81	2.00	1.15, 3.48	
Body mass index[b]										
<30	494	611	1.00	Referent	0.030	42	86	1.00	Referent	0.209
≥30	183	205	1.12	0.89, 1.42		62	86	1.54	0.91, 2.62	
Duration of oral contraceptive use, years										
≥1	355	503	1.00	Referent	0.119	44	103	1.00	Referent	0.245
<1	328	313	1.33	1.06, 1.67		60	69	1.74	0.99, 3.05	
Ever being pregnant										
Yes	575	734	1.00	Referent	0.052	91	164	1.00	Referent	0.079
No	108	82	1.49	1.06 2.08		14	8	2.43	0.88, 6.73	

Abbreviations: AF, attributable fraction; CI, confidence interval; OR, odds ratio.

[a] Adjusted for all of the variables in the table, as well as age, age at menarche, family history of breast or ovarian cancer, and breastfeeding.

[b] Weight (kg)/height $(m)^2$.

variables of interest in our analyses and dictated that we limit the number of potential confounders evaluated in our multivariable models. A larger sample would have afforded us the opportunity to further explore the effects of the timing and duration of oral contraceptive use and the timing of pregnancies or tubal ligation.

Additional limitations of our analysis included those related to the case-control method. The possibility of bias being introduced due to nonparticipation of ovarian cancer cases and controls should be considered. Although we used rapid case ascertainment to identify cases within 2 months of diagnosis and the median time to case interview was 4.5 months, which should have minimized survival bias, there is a possibility that cases who participated differed from those who did not. When we compared the tumor characteristics of ovarian cancer cases who were identified as eligible but did not participate (because of death, lack of physician consent, participant refusal, or inability to contact them) with the tumor characteristics of cases who did participate, we found that the proportion of invasive cases was slightly smaller among participants than among nonparticipants. This is not a surprising finding, given that cases who died quickly or for whom physicians did not give consent were more likely to have advanced disease. Among the invasive cases that were the focus of most of our analyses, we found no statistically significant differences in the proportions of higher-stage cancers between participants and nonparticipants. The racial/ethnic differences in histologic type that we observed among participants (i.e., a lower proportion of clear-cell tumors and a higher proportion of tumors of ''other'' histologic types among African Americans) were also observed among the nonparticipating cases. Thus, the invasive cases enrolled in the study appeared to be representative of the ovarian cancer cases diagnosed in our catchment area.

Nonparticipation also has the potential to introduce bias if participating cases and controls differ from persons who decline to participate in the study. Although we had no risk factor information on nonparticipants with which to assess their similarity with women who participated in the study, the associations we observed for white women within our study population are consistent with established ovarian cancer risk factors, which argues against our results' being biased due to nonparticipation.

Despite the limited sample of African-American women, the descriptive characteristics of our population and the attributable fraction analyses suggest that the relative importance of ovarian cancer risk factors may vary between African Americans and whites because of the substantial differences in the prevalence and strength of associations with factors such as tubal ligation and obesity. Tubal ligation, which had a stronger association with ovarian cancer among African Americans and is considerably more common among African Americans in our study population as well as in national surveys (6), could be an important explanatory factor for the lower rates of ovarian cancer among African Americans.

Obesity, which has shown modest associations with ovarian cancer risk in the majority of studies (22, 24–26), may be a considerably more important risk factor for African-American women, as evidenced by the markedly higher attributable fraction for obesity that we observed in our data. Consistent with national statistics (12), our data showed a much higher prevalence of obesity among African Americans than among whites. In particular, severe obesity

(BMI ≥35), which had a threefold higher prevalence among African Americans than among whites in our study, may be especially relevant as a risk factor for ovarian cancer among African Americans. Some investigators have reported either that associations between BMI and ovarian cancer risk were present only for persons with very high BMIs or that the relations were considerably stronger for women in the highest BMI categories (27, 28). Other investigators have found that the association between obesity and ovarian cancer was present only among premenopausal women or was much stronger for premenopausal ovarian cancer than for postmenopausal ovarian cancer (21, 22). Because the markedly higher prevalence of obesity among African-American women is apparent even among adults aged 20–39 years (12), African-American women may be at higher risk for ovarian cancer diagnosed at a younger age. This is consistent with the higher proportion of premenopausal ovarian cancer cases in African Americans as compared with whites (34% vs. 26%) and the younger mean age at diagnosis (54.8 years vs. 57.4 years) that we observed in our population and that has been reported in Surveillance, Epidemiology, and End Results data (1). The younger age at diagnosis also may be related to the stronger association with family history of breast or ovarian cancer among African-American women, which could be indicative of higher genetic risk.

Our data suggest that the relative importance of ovarian cancer risk factors may vary between African-American and white women because of differences in the prevalence of and strength of associations with characteristics such as tubal ligation, pregnancy, and obesity. However, conclusions that can be drawn from our data are limited by the small number of African Americans in our analysis, despite our study population's having more African-American women than any other existing study of ovarian cancer. Because ovarian cancer is a leading cause of cancer mortality in African Americans, there is a clear need for additional studies in order to deepen our understanding of causative and protective factors in this population.

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Exhibit 45

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Markers of inflammation and risk of ovarian cancer in Los Angeles County

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Factors that increase inflammation have been suggested to influence the development of ovarian cancer, but these factors have not been well studied. To further investigate this question, we studied the role of talc use, history of endometrioisis and use of non-steroidal anti-inflammatory drugs (NSAIDs) and risk of ovarian cancer in a population-based case-control study in Los Angeles County involving 609 women with newly diagnosed epithelial ovarian cancer and 688 population-based control women. Risk of ovarian cancer increased significantly with increasing frequency and duration of talc use; compared to never users risk was highest among long-duration (20+ years), frequent (at least daily) talc users (adjusted relative risk (RR) = 2.08, 95% confidence interval (CI) = 1.34–3.23). A history of physician-diagnosed endometriosis was statistically significantly associated with risk (RR = 1.66, 95% CI = 1.01–2.75). Women who were talc users and had a history of endometriosis showed a 3-fold increased risk (RR = 3.12, 95% CI = 1.36–7.22). Contrary to the hypothesis that risk of ovarian cancer may be reduced by use of NSAIDs; risk increased with increasing frequency (per 7 times/week, RR = 1.27, 95% CI = 1.14–1.43) and years of NSAID use (per 5 years of use, RR = 1.25, 95% CI = 1.10–1.42); this was consistent across types of NSAIDs. We conclude that risk of ovarian cancer is significantly associated with talc use and with a history of endometriosis, as has been found in previous studies. The NSAID finding was unexpected and suggests that factors associated with inflammation are associated with ovarian cancer risk. This result needs confirmation with careful attention to the reasons for NSAID use.

Key words: talc; endometriosis; non-steroidal anti-inflammatory drugs; ovarian cancer

In 1999, Ness and Cottreau proposed that chronic inflammation may lead to the development of epithelial ovarian cancer.[1] They hypothesized that factors including talc exposure, endometriosis and pelvic inflammatory disease (PID) may increase risk by a common pathway, increasing local inflammation of the "ovarian epithelium." They also suggested that studying the effect of non-steroidal anti-inflammatory drugs (NSAIDs) may offer additional opportunities to evaluate the inflammation hypothesis. In a 2008 paper, Merritt *et al.*[2] studied the role of inflammation, based on histories of talc use, PID, endometriosis and use of NSAIDs in the same study. They concluded that chronic inflammation is unlikely to play an important role because risk of ovarian cancer was modestly increased in association with talc use and history of endometriosis and was unrelated to use of NSAIDs but they restricted attention to medication use in the 5 years prior to diagnosis of ovarian cancer, rather than long-term use.[2] No support for the use of NSAIDs was found in a recent study conducted in Seattle, Washington which collected information on lifetime medication use. These investigators found increased risk of ovarian cancer in association with use of acetaminophen, aspirin and other NSAIDs, particularly among long (10+ years) term users.[3] We have conducted a population-based case-control study of ovarian cancer in Los Angeles County to further investigate the role of inflammation in the risk of ovarian cancer. We focused our attention on risk in relation to lifetime use of talc, NSAIDs and history of various gynecological conditions. We are particularly interested in risk patterns associated with long duration of NSAID use. We report our results herein.

Material and methods

Study design

This was a population-based case-control study of ovarian cancer. Eligible patients were English speaking residents of Los Angeles County between the ages of 18 and 74 inclusive who had histologically confirmed invasive or borderline (low malignant potential; LMP) ovarian cancers that were first diagnosed from 1998 to 2002. The cases were identified by the Cancer Surveillance Program (CSP), part of the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) Program, covering all residents of Los Angeles County.

A total of 1,097 patients meeting the pathological case definition were identified by the CSP. Of these, 136 patients had died or were too ill to be interviewed by the time we contacted them, 109 patients had moved away from Los Angeles County and could not be interviewed in person or they could not be located and 151 patients declined to be interviewed. Interviews were conducted with 701 ovarian cancer patients of whom 15 were later identified who did not have ovarian cancer and they were excluded from all analyses. Of the 686 ovarian cancer patients interviewed, 77 had a previous cancer (excluding non-melanoma skin cancer) before their diagnosis of ovarian cancer and were excluded from this report because their previous cancer diagnosis and/or treatment may have influenced use of NSAIDs and other risk factors. This left 609 ovarian cancer cases for the present analysis, 81% were invasive tumors [22% localized stage (Stage 1 or 2), 59% advanced stage (Stage 3 or greater) and 19% were LMP tumors. The cell type distribution is as follows: 58% serous, 14% clear cell/endometrioid, 12% mucinous and 16% other category.

Controls were identified through a well-established neighborhood recruitment algorithm, which we have used successfully in previous studies of breast, endometrial and other cancers to investigate the role of hormonal and non-hormonal medications and other factors.[4] For this study, controls were women with at least one intact ovary, with no history of cancer, except possibly non-melanoma skin cancer, and individually matched with patients on race/ethnicity (non-Hispanic White, African-American, Hispanic, Asians) and date of birth (+/−5 years). Neighborhood controls were sought by one of our staff who physically canvassed the neighborhood of the case using a systematic algorithm based on the address of the case. If the first eligible matched control declined to participate, the second eligible matched control in the sequence was asked, and so on. Letters were left when no one was at home, and follow-up by mail, telephone and further visits to the neighborhood continued until either an eligible control agreed to be interviewed or 150 housing units had been screened. When we failed to identify an exact race/ethnicity matched control, we

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accepted a control subject who was matched on age. A total of 688 control women were successfully interviewed by the closing date of the study. The first eligible match was interviewed for 76% of the patients, and the second match for another 17% and the third or later match for 6% of the patients. On average, we contacted a median number of 19 housing units to interview a matched control subjects for those neighborhoods with no refusal, a median of 36 housing units for those neighborhoods with 1 refusal and 58 housing units when there were 2 or more refusals.

Study participants were interviewed using a comprehensive questionnaire that covered medical, gynecological, reproductive and lifestyle history. All but 15 participants were interviewed in-person; cases and their matched controls were interviewed by the same person in almost all instances. A reference date was defined as 2 years before the date of diagnosis of the case. This same reference date was used for each case's matched control subject. Calendars were used to chart major life events and reproductive and contraceptive histories. Specifically, participants were asked if they were ever told by a physician that they had certain gynecological conditions including PID, gonorrhea, endometriosis, ovarian cysts, or uterine fibroids before the reference date. If the response was yes to any of the conditions, participants were then asked the age at which they were first diagnosed with the condition and if they had ever been treated for the condition. To determine the use of talcum powder, participants were asked if they ever used talc at least once per month for 6 months or more. If the response was positive, we then asked whether they had ever used talc in nonperineal areas (feet, arms, chest or back), perineal areas, or on underwear or sanitary pads/diaphragm. Questions on talc use included age at first use, frequency of use (times per month) and years of talc use. Few of the talc users (13 cases, 11 controls) had a tubal ligation or hysterectomy before they started using talc; the numbers were too sparse to determine for certain the effect of talc use in this group and these 24 users were included with the nonusers in subsequent analyses on frequency and duration of talc use. Results were unchanged when we excluded these 24 users from the analysis (data not shown).

We asked the participants whether they took prescription or nonprescription NSAIDs for various conditions including back trouble, arthritis, headaches, migraine headaches, dental problems, sinus trouble, colds or sore throats, menstrual pain or cramps or any other reason. They were also asked if they took any of these medications for prevention reasons, such as for prevention of heart attack. We explicitly asked about usage patterns of 10 common over-the-counter NSAIDs (regular aspirin, buffered aspirin, Anacin, APC, Tylenol, Excedrin, Advil, Nuprin, Coricidin, Dristan), 12 prescription brand-name NSAIDs (Clinoril, Motrin, Anaprox, Feldene, Empirin with codeine, Tylenol with codeine, Darvocet, Indocin, Fiorinal, Percocet-5, Percodan, Naprosyn) and two COX-2 inhibitors (Celebrex, Vioxx). We also asked the participants if they had used any NSAIDs that were not on our list and recorded the drug name and details of use. Respondents were also asked about use of 4 common diuretics; these medications are not hypothesized to be related to ovarian cancer risk, but they were included as a check of differential recall between cases and controls. Taking a specific medication 2 or more times a week for 1 month or longer was categorized as "use"; otherwise participants were considered "non-users." Participants were asked about the ages at first and last use, duration of use, usual frequency of use and the primary reason for such use. All of the medications data were categorized into the following groups based on their components: aspirin, acetaminophen, other NSAIDs, COX-2 inhibitors and diuretics.

Total duration and frequency of the main classes of medication (aspirin, acetaminophen, other NSAIDs) were calculated by summing all use of the same class of medication for each person (there were few users of COX-2 inhibitors, thus results are not shown). We also created a combined variable representing use of all NSAIDs. Duration of use was categorized as no use, less than 5 years, 5–10 years and >10 years of use of the specific type of medication (years of use of different medications may be overlapping). The no use category included never users, occasional users and those who only started to use a particular medication within the interval beginning 2 years before date of diagnosis for case patients and the same reference period for controls to avoid including medication use because of early symptoms in cancer patients. We also repeated the analyses excluding first use of medication within 5 years of diagnosis. In addition, we evaluated effect modification of the NSAIDs-ovarian cancer association by race/ethnicity, education, menopausal status, tumor stage, history of endometriosis, talc use and frequency of Pap smears in the 10 years before reference date.

TABLE I – DEMOGRAPHIC AND OTHER CHARACTERISTICS OF OVARIAN CANCER PATIENTS AND CONTROLS

	Cases $N = 609$	Controls $N = 688$	RR	95% CI[1]
Race/ethnicity				
Non-Hispanic White	381	503		
Black	41	44		
Hispanic	136	103		
Asian	51	38		
Age				
≤34	40	36		
35–44	92	138		
45–54	162	227		
55–64	149	162		
65+	166	125		
Education				
≤high school	92	50		
Some college	109	81		
College graduate	223	242		
Graduate	185	315		
Family history of ovarian cancer				
No	581	672	1.00	
Yes	26	16	1.76	0.89–3.47
p-value			0.10	
Number of livebirth				
0	156	149	1.00	
1	98	110	0.76	0.52–1.12
2	157	202	0.61	0.43–0.86
3	109	118	0.61	0.41–0.90
4+	89	109	0.34	0.22–0.53
p trend			<0.0001	
Oral contraceptives				
0 yr	241	189	1.00	
>0 to <5 yr	259	261	0.98	0.73–1.32
≥5 to <10 yrs	57	112	0.54	0.36–0.82
≥10 yrs	52	126	0.40	0.26–0.61
p trend			<0.0001	
Tubal ligation				
No	531	553	1.00	
Yes	78	135	0.66	0.47–0.93
p value			0.017	

[1]Adjusted for race/ethnicity, age, education, tubal ligation, family history of ovarian cancer, menopausal status, use of oral contraceptives, and parity.

The study was approved by the Institutional Review Board of the Keck School of Medicine at the University of Southern California. Informed consent was obtained from each case and control before her interview.

Statistical methods

We calculated odds ratios as estimates of relative risk (RR), their corresponding 95% confidence intervals (CIs) and statistical significance (p) values. We used conditional stratified logistic regression analysis, with stratification sets defined jointly by age (<35, 35–44, 45–54, 55–64, ≥65) and race/ethnicity (non-Hispanic White, African-American, Hispanic, Asians). All regression models also included as categorical covariates education level (high school or less, some college, college graduate, >college),

TABLE II – MULTIVARIABLE RRS (95% CIS) FOR TALC USE AND RISK OF OVARIAN CANCER

	Cases	Controls	RR	95% CI[1]
Talc use				
No[2]	363	469	1.00	
Yes	242	219	1.48	1.15–1.91
Yes, non-perineal area[3]	112	103	1.43	1.03–1.98
Yes, perineal area	130	116	1.53	1.13–2.09
Frequency and duration of talc use				
No	363	469	1.00	
1 ≤20 yrs and ≤10 times/month	35	31	1.36	0.79–2.32
1 ≤20 yrs and >10 to ≤30 times/month	23	30	1.16	0.63–2.12
1 ≤20 yrs and >30 times/month	21	21	1.23	0.63–2.41
>20 yrs and ≤10 times/month	45	49	1.27	0.80–2.01
>20 yrs and >10 to ≤30 times/month	51	43	1.57	0.99–2.50
>20 yrs and >30 times/month	67	45	2.08	1.34–3.23
p (6 df)				$p = 0.032$
Total times of talc use				
No	363	469	1.00	
≤5200	49	52	1.20	0.77–1.88
>5200 to ≤15600	46	47	1.38	0.87–2.20
>15,600 to ≤52000	63	61	1.34	0.89–2.02
>52000	84	59	1.99	1.34–2.96
p (1 df)				$p = 0.0004$
Total times of talc use				
No	363	469	1.00	
Before 1975				
≤5200	24	35	0.84	0.47–1.51
>5200 to ≤15600	29	29	1.41	0.79–2.53
>15,600 to ≤52000	49	45	1.45	0.91–2.31
>52000	82	58	1.93	1.29–2.88
After 1975				
≤5200	25	17	1.95	0.98–3.89
>5200 to ≤15600	17	18	1.17	0.56–2.48
>15,600	16	17	0.98	0.45–2.13

[1]Adjusted for race/ethnicity, age, education, tubal ligation, family history of breast/ovarian cancer, menopausal status, use of oral contraceptives and parity.–[2]Subjects (13 Cases, 11 Controls) reported tubal ligation and/or hysterectomy before started talc use and were included with the never users.–[3]Included arms and extremities.

age at menarche (<=11, 12, 13, 14+), parity (0, 1, 2, 3, 4+ births), use of oral contraceptives (none, >0 to <5, 5 to <10, 10+ years), family history of breast/ovarian cancer (no/yes), menopausal status (premenopausal, natural or surgical menopause) and tubal ligation (no/yes). Results obtained using stratified conditional logistic regression methods were consistent with those obtained in matched analyses that preserved the original case-control matching, and we show the results from the stratified analyses. All statistical significance p values quoted are two-sided and are standard chi-squared tests based on differences in log-likelihoods.

Results

The race/ethnicity, age and education of the ovarian cancer cases and controls are shown in Table I. Risk of ovarian cancer increased in association with family history of ovarian cancer (RR = 1.76, 95% CI = 0.89–3.47) and decreased significantly with increasing number of births (RR per birth = 0.79, 95% CI = 0.72–0.88), with increasing duration of oral contraceptive use (RR per 5 years of use = 0.73, 95% CI = 0.64–0.83) and with a history of tubal ligation (RR = 0.66, 95% CI = 0.47–0.93).

Table II shows risk associations with talc use. Ever use of talc was associated with a statistically significant increased risk (RR = 1.48, 95% CI = 1.15–1.91). This included talc that was applied to the perineal area (RR = 1.53, 95% CI = 1.13–2.09) and to the nonperineal area only (RR = 1.43, 95% CI = 1.03–1.98). Elevated risks were found among those who used talc on sanitary napkins (RR = 1.61, 95% CI = 0.93–2.78), underwear (RR = 1.71, 95% CI = 0.99–2.97) and on diaphragm/cervical caps (RR = 1.14, 95% CI = 0.46–2.87). When we examined risk patterns by frequency and duration of talc use, the effect of frequency of use was relatively modest among users of less than 20 years but there was a clear trend of increasing risk with increasing frequency of use among longer duration (>20 years) users. Compared with never users, risk was highest in long-term (>20 years), daily (>30 times/month) talc users (RR = 2.08, 95% CI = 1.34–3.23). Risk increased significantly with lifetime total times of talc use, but the association was limited to those who started talc use before 1975 (p_{trend} <0.001). The association between talc use and risk of ovarian cancer was strongest for serous ovarian cancer, the RR associated with any use was 1.70 (95% CI = 1.27–2.28). The risk associations for talc use and other histologic cell types overlapped with the finding for serous ovarian cancer (RRs were 0.99 for mucinous, 1.19 for clear/endometrioid and 1.46 for other cell types). Elevated risks in relation to talc use were found for those with invasive cancers (RR = 1.31, 95% CI = 0.85–2.01 for localized stage; RR = 1.66, 95% CI = 1.22–2.26 for advanced stage) and LMP tumors (RR = 1.32, 95% CI = 0.88–2.22).

Women with a history of physician-diagnosed endometriosis experienced a nearly 2-fold increased risk of ovarian cancer (RR = 1.95, 95% CI = 1.20–3.17). The risk associated with endometriosis remained statistically significant after adjustment for other gynecological conditions including PID, gonorrhea, ovarian cysts and uterine fibroids (adjusted RR = 1.66, 95% CI = 1.01–2.75) (Table III). Small (4–18%) increased risks were also associated with a history of the other gynecological conditions as mentioned earlier but none of these findings were statistically significant (data not shown). The risk of ovarian cancer increased significantly (RR = 2.58) for more recent diagnoses of endometriosis (2–10 years prior to cancer diagnosis) and was less strong (RR = 1.58) for women with diagnosis more than 10 years previously. The endometriosis-risk association was stronger for invasive cancers (RR = 1.80, 95% CI = 0.85–3.80 for localized stage, RR =

TABLE III – MULTIVARIABLE RRS (95% CIS) FOR PREVIOUS OVARIAN DISEASE AND RISK OF OVARIAN CANCER

		Cases	Controls	Adjusted RR[1]	Adjusted RR[2]
Pelvic inflammatory disease					
No		579	657	1.00	1.00
Yes		25	22	1.48 (0.78–2.82)	1.15 (0.60–2.21)
Gonorrhea					
No		553	619	1.00	1.00
Yes		51	60	1.19 (0.77–1.84)	1.04 (0.67–1.62)
Endometriosis					
No		553	642	1.00	1.00
Yes		51	37	1.95 (1.20–3.17)	1.66 (1.01–2.75)
Years since first diagnosed					
2–10		15	8	2.66 (1.06–6.64)	2.58 (1.03–6.48)
11+		36	29	1.56 (0.90–2.70)	1.58 (0.91–2.76)
Talc	Endometriosis				
No	No	332	435	1.00	1.00
No	Yes	29	28	1.68 (0.93–3.04)	1.67 (0.92–3.01)
Yes	No	221	207	1.50 (1.15–1.94)	1.49 (1.15–1.94)
Yes	Yes	22	9	3.17 (1.38–7.29)	3.12 (1.36–7.22)
p (3df)					0.001

[1]Adjusted for race/ethnicity, age, education, tubal ligation, family history of breast/ovarian cancer, menopausal status, use of oral contraceptives and parity.–[2]Adjusted for other conditions including pelvic inflammatory diseases, gonorrhea, endometriosis, ovarian cyst and fibroids.

1.87, 95% CI = 1.04–3.35 for advanced stage) than for LMP tumors (RR = 1.28, 95% CI = 0.56–2.95). Although the point risk estimate was slightly higher for clear/endometrioid cancers (RR = 1.97), the risk associations for the other cell types were all around 1.70. Compared with women who did not have endometriosis and were nontalc users, risk increased 3-fold (RR = 3.12, 95% CI = 1.36–7.22) in women who had endometriosis and were talc users whereas about 50% increased risk was observed in women who had either exposure.

Risk of ovarian cancer increased significantly with increasing duration and frequency of use of all NSAIDs (*i.e.*, aspirin, acetaminophen, other NSAIDs). The risk per 5 years of NSAID use was 1.25 (95% CI = 1.10–1.42) and the risk per 7 times of NSAID use per week was 1.27 (95% CI = 1.14–1.43). The effect of total pill use was essentially identical to the effect of frequency of use. This pattern of risk elevation was found for aspirin, acetaminophen and other NSAIDs although the results were statistically significant only for other NSAIDs (Table IV). Risks patterns remained essentially unchanged when we adjusted for indication of use (*i.e.*, headaches, back pain, menstrual pain and others) or history of endometriosis and other gynecological conditions. Risk associations were only slightly reduced when we restricted our analyses to medication use at least 5 years before diagnosis; the RR per 5 years of all NSAID use was 1.20 (95% CI = 1.06–1.43) and the risk per 7 times of NSAID use per week was 1.23 (95% CI = 1.09–1.38). In contrast, risk of ovarian cancer was not significantly related to duration or frequency of use of diuretics (RRs were 1.00, 1.39, 0.89, 0.65, respectively for no, 1–5, >5–10, >10 years of use, $p_{trend} = 0.50$).

Table V presents stratified results, when we performed a series of analyses to evaluate whether the findings with NSAID use were consistent across levels of various subgroups of interest including race/ethnicity, education, menopausal status, tumor stage, endometriosis, talc use, use of oral contraceptives, parity and frequency of Pap smears in recent 10 years as a marker of access to care. Elevated risks in relation to NSAID use were found in all the subgroup analyses; findings were similar by race/ethnicity, menopausal status, talc use, oral contraceptive use, parity and history of Pap smear. There were some differences in risk estimates by education, tumor stage, history of endometriosis but they were not statistically significantly different. We considered these differences by tumor stage, history of endometriosis and education in our interpretation of these results.

Discussion

The main objective of this population-based case-control study was to comprehensively investigate the role of inflammation in risk of ovarian cancer by studying factors that have been hypothesized to increase inflammation (*e.g.*, talc, endometriosis) or to reduce inflammation (NSAIDs) simultaneously in the same population. Our findings on talc and endometriosis are consistent with previous findings and are compatible with the hypothesis that these factors increase the risk of ovarian cancer and that inflammation may be a common pathway.[1,2,5] However, contrary to the study hypothesis that NSAIDs may have chemopreventive effects by decreasing inflammation,[6] we found that risk of ovarian cancer increased significantly with increasing frequency and duration of NSAIDs use.

Our results on NSAID and risk are similar to the recent results reported in the population-based case-control study conducted in Seattle, Washington.[3] In both studies, women were asked to recall prescription and nonprescription medications taken over their lifetime for various conditions. In the Seattle study, risk of ovarian cancer increased significantly in association with 10+ years of use of acetaminophen (RR = 1.8, 95% CI = 1.3–2.6), aspirin (RR = 1.6, 95% CI = 1.1–2.2) and other NSAIDs (RR = 1.3, 95% CI = 1.0–1.7).[3] Mechanisms whereby use of NSAID may increase risk of ovarian cancer may be related, in part, to the underlying conditions associated with medication use.

However, our results and those from the Seattle study differed from most previous studies on this topic. As Cramer *et al.* reported risk reduction of ovarian cancer with ever use of aspirin, and acetaminophen, but not with use of ibuprofen,[7] 7 (3 case-control, 4 cohort) of 13 (7 case-control, 6 cohort) studies have found no significant relation with use of NSAID. The case-control studies showing null findings were conducted in Italy,[8] the UK[9] and Australia,[2] and they investigated risk associations with use of aspirin,[8] acetaminophen and other NSAID,[9] and aspirin and other NSAIDs,[2] respectively. There was also no relationship between acetaminophen use and risk in the Cancer Prevention II Mortality Study[10] or between risk and use of low-dose aspirin[11] and other NSAIDs[12] in a Danish prescription database study. In the Breast Cancer Detection Demonstration Project Follow-up Study (BCDDP), risk was not significantly related to use of aspirin, acetaminophen and other NSAIDs but risk was increased with 5+ years of other NSAID use (RR = 2.0, 95% CI = 0.95–4.2).[13] Six other studies (4 case-control, 2 cohort) are supportive of an inverse

TABLE IV – MULTIVARIABLE RRS[1] (95% CIS) FOR USE OF ALL NSAIDs (ASPIRIN, ACETAMINOPHEN, OTHER NSAIDs) AND RISK OF OVARIAN CANCER

	Excluded medication use the 2 years before reference date		
	Cases	Controls	RR (95% CI)
All NSAIDs			
Years of use			
Never[2]	355	486	1.00
1 to 5 yr	117	99	1.71 (1.23–2.39)
>5 to ≤10 yr	37	33	1.59 (0.93–2.72)
>10 yr	79	57	1.81 (1.21–2.71)
p trend			<0.001
No. of pills per week			
Never[2]	355	486	1.00
1 to ≤7/wk	82	66	1.62 (1.11–2.39)
>7 to ≤14/wk	41	49	1.09 (0.67–1.78)
>14/wk	110	74	2.24 (1.56–3.21)
p trend			<0.001
Total no. of pills			
Never	355	486	1.00
1 to ≤1096	73	63	1.60 (1.08–2.38)
>1096 to 6428	73	66	1.43 (0.96–2.13)
>6428	87	60	2.22 (1.49–3.31)
p trend			<0.001
Years of use by type[3]			
Aspirin			
Never[2]	492	597	1.00
1 to 5 yr	46	25	2.13 (1.21–3.77)
>5 to ≤10 yr	13	18	0.70 (0.31–1.58)
>10 yr	31	28	1.15 (0.62–2.13)
p trend			0.43
Acetaminophen			
Never[2]	491	590	1.00
1 to 5 yr	47	53	0.87 (0.53–1.41)
>5 yr	44	25	1.71 (0.94–3.09)
p trend			0.12
Other NSAIDs			
Never[2]	450	575	1.00
1 to 5 yr	87	61	1.76 (1.18–2.63)
>5 to ≤10 yr	17	19	1.18 (0.55–2.53)
>10 yr	28	13	2.18 (1.03–4.63)
p trend			0.008
Frequency of use by type[3]			
Aspirin			
Never[2]	492	597	1.00
1 to ≤7/wk	61	48	1.49 (0.94–2.35)
>7	29	23	1.18 (0.61–2.29)
p trend			0.21
Acetaminophen			
Never[2]	491	590	1.00
1 to ≤7/wk	48	45	1.04 (0.63–1.71)
>7 /wk	43	33	1.36 (0.78–2.36)
p trend			0.33
Other NSAIDs			
Never[2]	450	575	1.00
1 to ≤7/wk	52	38	1.56 (0.95–2.56)
>7 to ≤14/wk	29	25	1.27 (0.68–2.40)
>14/wk	51	30	2.22 (1.30–3.79)
p trend			0.0009

[1]Adjusted for age, education, race, tubal ligation, family history of breast/ovarian cancer, menopausal status, use of oral contraceptives, parity and talc use.–[2]Included participants who started medication within 2 years of diagnosis/reference date.–[3]Additional adjustment for history of PID, gonorrhea, ovarian cysts, endometriosis, and fibroids. The RRs for aspirin, acetaminophen and other NSAIDs were mutually adjusted. Aspirin included regular aspirin, buffered aspirin; acetaminophen included Tylenol, coricidin, Dristan, darvocet, Percocet, Excedrin; other NSAID included advil, nurin, clinoril, motrin, anaprox, feldene, indocin, naprosyn.

association with NSAIDs use, one reported significant risk reduction with acetaminophen use[14] while 4 studies found significant reduced risk with use of other NSAIDs[15–18] but there were differences in these results. In one study, an inverse association was found only in nulliparous and nonoral contraceptive users.[18] No dose-response relationship was observed in a second study,[15] and information on NSAID use was limited to the 5 years before diagnosis in a third study.[17] Aspirin use was not significantly associated with risk in these 5 studies.[14–17,19] Ascertainment of NSAID use was heterogeneous in these studies: different NSAIDs were included, the exposure period varied (*e.g.*, adult use, use in previous 20 years or previous 5 years before ovarian cancer diagnosis), and information on frequency and duration of NSAID use was asked in only some studies.

An advantage of our study is that we collected detailed information on adult usage history of both over the counter and prescription NSAIDs including duration and frequency of use and indication for use. Our results suggest increased risk associated with duration and frequency of use of aspirin, acetaminophen and other NSAIDs although only the findings for other NSAIDs were statistically significant on their own. We adjusted for potential confounders and indication for use; the latter was considered in only some previous studies. Nevertheless, our results should be interpreted with caution for the following reasons. Our assessment of NSAID use was based on self-report without assessment of reliability of recall. However, a drug validation study conducted by colleagues in Los Angeles County found high and comparable concordance rate of recall of analgesics in cancer patients and control subjects.[20] Regular NSAID use was reported by 29% (31% in non-Hispanic whites) of controls in our study; comparable with the rate reported in Wisconsin and Massachusetts (34%)[18] but lower than that in Seattle (41%).[3] Differences in the assessment of use of NSAID complicate comparison of prevalences of use between studies.

Although an increased risk was specific to NSAIDs use and no increased risk was found with diuretic use, we cannot rule out the possibility of selective recall bias among ovarian cancer cases. Given that many NSAIDs products are available and use may be episodic, it is conceivable that some cases may be more motivated to remember their NSAID use than control subjects. There is also the possibility of surveillance bias and that certain health conditions led to regular NSAID use, resulting in frequent doctor visits, which increased the chances of ovarian cancer detection. As noted earlier, the prevalence of NSAID use was higher in women with LMP tumors or localized cancer than those with advanced stage cancers, and the magnitude of association was stronger for earlier stage cancers. However, the proportion of LMP/localized stage cancers among those we interviewed (41%) and those we failed to interview (39%) was not dissimilar, suggesting there should be minimal overestimation of the overall effect of NSAID in relation to this reason. There also may be residual confounding by indication for use. Another possible explanation for our observed positive finding is that women with early symptoms of undiagnosed ovarian cancer take pain medications to relieve these symptoms. This seems less likely because our results were essentially unchanged when we excluded participants who first started using these medications within the 5 years of diagnosis. Finally, we consider possibly that selection bias of cases and controls may have affected our finding. Our response rate was modest; cases who participated may differ from those who did not participate. Although controls in our study had more years of education than cases, there was no consistent pattern in the NSAID-risk association by education. The NSAID-risk association was most apparent in women who were college graduates but was very similar in women with high school education or less and those who had more than college education. Thus, despite these limitations, our results raise the concern that NSAIDs, taken as aspirin, acetaminophen or other NSAIDs, may actually increase the risk of ovarian cancer.

In our study, history of self-reported history of endometriosis that was diagnosed by a physician was associated with a significant 66% increased risk of ovarian cancer. Given that the elevated risk was observed for those with previous endometriosis for at least 11+ years, it is unlikely that our finding is due to detection bias but suggests that endometriosis may have an etiological role.

TABLE V – PREVALENCE OF NSAID USE IN CASES AND CONTROLS AND RRS (95% CI)[1] PER 5 YEARS OF NSAID USE

		ever NSAID-cases (%)	ever NSAID-controls (%)	10+ yrs of NSAIDs-cases (%)	10+ yrs of NSAID-controls	RR (95% CI) per 5 years of NSAID
Race/ethnicity	Non-Hipsanic Whites	45%	31%	17%	10%	1.23 (1.07–1.42)
	Other	31%	19%	7%	4%	1.37 (1.03–1.84)
Education	<College	36%	29%	14%	10%	1.09 (0.84–1.41)
	College graduate	48%	27%	16%	7%	1.71 (1.36–2.15)
	Graduate	33%	28%	11%	9%	1.05 (0.85–1.30)
Menopause	Premenopause	34%	22%	7%	5%	1.35 (1.05–1.73)
	Postmenopause	43%	34%	17%	11%	1.22 (1.06–1.42)
Tumor stage	LMP	47%	28%	14%	8%	1.37 (1.11–1.69)
	Invasive, Stage 1 or 2	40%	28%	14%	8%	1.36 (1.11–1.67)
	Invasive, Stage ≥3	37%	28%	13%	8%	1.16 (1.01–1.35)
Endometriosis	No	39%	27%	12%	9%	1.23 (1.08–1.40)
	Yes	50%	44%	26%	8%	1.52 (0.96–2.43)
Talc	No	36%	25%	13%	6%	1.31 (1.10–1.55)
	Yes	45%	35%	15%	14%	1.14 (0.94–1.38)
Oral Contraceptives	No	35%	25%	13%	10%	1.10 (0.89–1.37)
	Yes	43%	29%	14%	8%	1.31 (1.12–1.53)
Parity	No	41%	31%	15%	9%	1.28 (0.98–1.68)
	Yes	40%	27%	13%	8%	1.25 (1.09–1.45)
Pap smear[2]	≤5 times	34%	24%	11%	7%	1.22 (0.94–1.59)
	>5 times	42%	30%	15%	9%	1.27 (1.09–1.47)

[1]Adjusted for age, education, race, tubal ligation, family history of breast/ovarian cancer, menopausal status, use of oral contraceptives and parity.–[2]Frequency of Pap smears in the 10 years before reference date.

No association between endometriosis and ovarian cancer was reported in the Iowa Women's Health Study, but this may be because of the relatively limited number of ovarian cancers in this cohort and the low prevalence of endometriosis (~3%).[21] Endometriosis was associated with about a 30% increased risk in an Australian population-based case-control study[2] and in a pooled analysis of 2,098 cases and 2,953 controls from 4 US population-based case-control studies.[22] Although the prevalences of endometriosis among cases (8.4%) and controls (5.4%) in our study are very comparable with the figures reported in cases (8%) and controls (6%) in previous case-control studies,[2,22] a limitation of our study and other case-control studies on this topic is that history of endometriosis is not validated. We did not see meaningful differences in history of endometriosis by cell type (11% for endometrioid/clear cell *vs.* 8% for other cell types) of ovarian cancer while a higher prevalence of endometriosis in women with endometroid/clear cell has been usually reported in other studies.[2,23] Interestingly, when one of us (CT) reviewed the pathology reports of the 52 ovarian cancer patients who reported a history of endometriosis, endometriosis in the ovary was documented in only 15 patients (15 of 604 cases = 2.5%) but the percent was higher in women with clear cell/endometrioid (7 of 84 = 8.3%) ovarian cancer compared with the other cell types (8 of 520 = 2.3%). Additional information on the type of endometriosis and location of endometriosis would be helpful in future studies.

The role of talc in the development of ovarian cancer has been studied extensively. In a 2006 review by the International Agency for Research on Cancer (IARC), talc was classified as possibly carcinogenic to humans (*i.e.*, Group 2B) on the basis that most of the 20 epidemiological studies on talc and ovarian cancer show consistently a 30–60% increased risk associated with talc use.[24] However, only about half of the studies examined exposure-response relationships and the evidence for this is less consistent. Our study adds to the small group of studies that have investigated the combination of frequency and duration of talc use on ovarian cancer risk.[25–28] Our results show a significant trend with increasing number of total applications. Using a combined index of total applications or cumulative lifetime days of talc use, 2 studies showed a higher risk with greater exposure[27,29] but this was not observed in 2 other studies.[25,28] When we investigated the combined effect of frequency and duration, our results suggest that the effect of increasing frequency was modest in users of less than 20 years but that the effect of frequency was clearer in women who had used talc for 20 years or more. Our results also suggest that talc use prior to 1976 may be more important. In 1976, talcum powder manufacturers instituted voluntary guidelines to prevent asbestos contamination in talc products and thus formulations after 1976 may be less likely to be contaminated with asbestos fibers. Stronger associations with talc use in the 1960s and 1970s have been reported in some studies[25,27] but not in others.[2,28] Thus, lack of sufficient information on frequency, duration and calendar period of talc use may have contributed to misclassification of this exposure variable in some previous studies.

Our findings on talc use and endometriosis and ovarian cancer risk are compatible with previous studies. However, the NSAID finding in this study was unexpected and requires confirmation with further characterization of the association by frequency and duration of use, cumulative dose and timing of exposure. In addition, it will be important to evaluate the underlying conditions for medication use.

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Exhibit 46

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Genital powder exposure and the risk of epithelial ovarian cancer

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Abstract

Background—We conducted a population-based, case–control study to examine the association between the use of genital powder and ovarian cancer risk, including measures of extent and timing of exposure. We also assessed the relationship of powder use with risk of disease subtypes according to histology and degree of malignancy.

Methods—Information was collected during in-person interviews with 812 women with epithelial ovarian cancer diagnosed in western Washington State from 2002 to 2005 and 1,313 controls. Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs).

Results—Overall, the perineal use of powder after bathing was associated with a slightly increased ovarian cancer risk (OR = 1.27, 95% CI: 0.97–1.66), which was most evident among women with borderline tumors (OR = 1.55, 95% CI: 1.02–2.37). We noted no clear pattern of risk increase on the basis of the extent of use, assessed as years in which powder was used, or as lifetime number of applications for invasive or borderline tumors, or their histologic subtypes. There was no alteration in the risk of ovarian cancer associated with other types of powder exposure (e.g., on sanitary napkins or diaphragms).

Conclusions—The International Agency for Research on Cancer has designated perineal exposure to talc (via the application of genital powders) as a possible carcinogen in women. A modest association of ovarian cancer with this exposure was seen in our study and in some previous ones, but that association generally has not been consistent within or among studies. Therefore, no stronger adjective than “possible” appears warranted at this time.

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Keywords

Ovarian neoplasms; Talc; Epidemiology

Introduction

Talc deposition in the body can lead to inflammatory and neoplastic changes [1], and perineal exposure to certain particulates (conceivably, talc) can lead to their deposition in the peritoneal cavity and ovaries [2]. Multiple case–control studies [3-5] and one cohort study [6] have examined risk of ovarian cancer associated with perineal exposure to dusting powders (many of which contain talc), either overall or within specific histologic subgroups of disease. In a meta-analysis of 20 case–control studies [3], the summary estimate of the relative risk (RR) of ovarian cancer among women who reported any perineal use of powder was 1.35 (95% confidence interval (CI): 1.26–1.46). Although no association of this exposure with ovarian cancer risk was noted in the cohort study overall, risk of invasive serous cancers was modestly elevated (by 40%). The International Agency for Research on Cancer has classified perineal exposure to talc as group 2B (possibly carcinogenic to humans [3, 7]).

In this article, we describe the results of a large population-based study of epithelial ovarian cancer conducted in western Washington State in which we further investigated the association between the use of genital powder and the ovarian cancer risk, including measures of extent of use and aspects of timing of exposure. We also assessed the relationship of powder use with risk of disease subtypes according to histology and degree of malignancy.

Materials and methods

The study population and methods have been described previously [8]. Female residents, of a 13 county areas of western Washington State, 35–74 years of age, who were diagnosed with a primary invasive or borderline (also known as low malignant potential or LMP) epithelial ovarian tumor between 1 January 2002 and 31 December 2005, were considered eligible as cases. The cases were identified through a population-based cancer registry, the Cancer Surveillance System, which is part of the Surveillance, Epidemiology, and End Results Program (SEER) of the US National Cancer Institute. We restricted our cases to English-speaking women who had residential telephones at the time of diagnosis, because random digit dialing (RDD) was the method used to select control subjects. Of the 1,058 eligible women identified, 812 (76.6%) were interviewed. Of the interviewed cases, 595 had invasive disease. Tumors were categorized into the following histologic subgroups: serous (n = 452), mucinous (n = 112), endometrioid (n = 104), clear cell (n = 35), and other epithelial tumors (n = 109).

Controls were selected by RDD using stratified sampling in 5-year age categories, 1-year calendar intervals, and two county strata in a 2:1 ratio to women with invasive cancer. For 14,561 (82.0%) of the 17,768 telephone numbers belonging to residences, we determined whether an eligible woman (i.e., an age and county eligible woman able to communicate in English and, if so, with at least one ovary and no prior history of ovarian cancer) resided there. Of the 1,561 eligible women identified, 1,313 were interviewed (84.1%) for an overall (screening X interview) response proportion of 69%.

The study was approved by the Institutional Review Boards of the Fred Hutchinson Cancer Research Center and the University of Illinois at Urbana Champaign, and all women

provided signed informed consent before participating. In-person interviews pertained to the period of time before diagnosis (for cases) or before an assigned comparable reference date (for controls), and covered the following: demographic and lifestyle characteristics; medical history; and detailed reproductive history, including menstrual, pregnancy, and contraceptive history, as well as the use of contraceptive and menopausal hormone preparations. To aid recall, interviewers used a calendar to record major life events and provided photographs of the commonly used oral contraceptive and menopausal hormone preparations.

Several sources of genital powder exposure were assessed in separate questions, including direct perineal application after bathing, its use on sanitary napkins and contraceptive diaphragms, and the use of feminine (vaginal) deodorant spray. For powder use on sanitary napkins and use of feminine deodorant sprays, we recorded the total number of months or years in which these products were used (with a minimum of at least 1 month of regular use). For the use of powder on the perineum after bathing, only intervals of at least 1 year when powder was usually used were recorded. For each reported interval in which powder was usually used on the perineum after bathing, we recorded the age when began and ended, the number of weeks or months of use per year, and the average days per week used. Women were also asked to report the types of powder(s) used after bathing, including talcum, baby, cornstarch, deodorant, body/bath, and other or unknown. The extent of exposure to perineal powder after bathing was assessed as lifetime duration of use (i.e., total number of years in which this exposure occurred), and as the estimated lifetime number of applications (i.e., a measure that incorporated both the duration and frequency of use).

Using unconditional logistic regression, we calculated odds ratios (ORs) and related 95% confidence intervals (CIs) as estimates of the RR of epithelial ovarian cancer associated with various aspects of genital powder use. All the analyses were adjusted for the frequency-matching variables of age (5-year intervals), county of residence (dichotomized as the three urban or the 10 rural/suburban counties in the study), and calendar year of diagnosis/reference date (continuous), as well as number of full-term pregnancies (0, 1, 2, or ≥3), and duration of hormonal contraception (Never,<6, 6–59, 60–119, or ≥120 months). Additional adjustment for other potential confounding variables, including race/ethnicity, education, age at menarche, body mass index (BMI), smoking, alcohol drinking, family history of breast or ovarian cancer, personal history of breast cancer, endometriosis, tubal ligation, hysterectomy, unilateral oophorectomy, and the use of menopausal hormone therapy, produced no important change in the OR estimates. We used polytomous logistic regression to examine risk among subgroups of case women with borderline and invasive tumors and in women with different histologic subtypes of these tumors. Because we had limited facility to separately examine risk of mucinous invasive ovarian cancer owing to its rarity ($n = 23$), we excluded these tumors when examining histologic subtypes of invasive disease; similarly, we limited our examination of histologic subtypes of borderline tumors to serous or mucinous subtypes (excluding 11 women with other, uncommon histologic subtypes of borderline tumors). All the analyses were carried out using the STATA version 10 statistical package (Statacorp LP, College Station, Texas).

Results

Characteristics of cases and controls have previously been described [8, 9]. Approximately 90% of cases and controls were non-Hispanic white women. Cases were less likely than controls to have given birth, and reported a lesser extent of exposure to hormonal forms of contraception. Cases were somewhat more likely than controls to be overweight (BMI 25–<30 kg/m^2) or obese (BMI ≥ 30 kg/m^2), and less likely to have graduated from college (results not shown).

The use of powder after bathing (for at least 1 year of regular use, as described above) was reported by approximately 12% of controls (Table 1). Other sources of powder exposure (e.g., on sanitary napkins) and the use of deodorant sprays were reported by a slightly smaller proportion of women, despite the shorter minimum time interval allowed in our assessment of these exposures (see Methods for description). Overall, the perineal use of powder after bathing was associated with a slightly increased risk (OR = 1.27, 95% CI: 0.97–1.66), which was the most evident among women with borderline tumors (OR = 1.55, 95% CI: 1.02–2.37). No clear elevation in risk of borderline or invasive tumors were observed in association with the use of powders on sanitary napkins or contraceptive diaphragms, or with the use of feminine deodorant sprays (Table 1). The most frequently reported category of product used after bathing was baby powder (not shown); few women reported exclusive use of talcum powder or of cornstarch (a product that does not contain talcum powder). Within limits of precision, findings regarding ovarian cancer risk among women who reported the use of talcum powder were similar to those presented for all types of powders combined; e.g., the risk of invasive ovarian cancer among women who reported the use of talcum powder was 1.38 (95% CI: 0.77–2.47).

We noted no evidence that risk of ovarian cancer increased in association with increasing extent of the use of perineal dusting powder (assessed as years in which powder was used or as lifetime number of applications) for either invasive or borderline tumors (Table 2). Similarly, we observed no trend in risk with increasing years of powder use on sanitary napkins or with the use of feminine deodorant sprays (results not shown). Risk (relative to never-users) was increased among women who first reported the regular use of perineal dusting powders at age 30 years or older (OR for invasive and borderline tumors combined = 1.69, 95% CI: 1.08–2.64), among women whose first regular use was in 1980 or later (OR for invasive and borderline tumors combined = 2.03, 95% CI: 1.28–3.24), and among women who had initiated regular use within the last 25 years (cut-off point based on approximate quartiles of exposed controls; OR for invasive and borderline tumors combined = 1.77, 95% CI: 1.12–2.78). Point estimates for each of these exposure subgroups were similar for borderline and invasive tumors (Table 2).

We repeated our analyses after [1] restricting the analysis to the use of perineal powder that occurred before tubal ligation and/or hysterectomy (for women who had undergone those procedures) and [2] restricting it to the use of powder that occurred at age 15 years or later. Results were generally similar to those that we have presented. Associations with any perineal powder exposure that occurred in women with intact fallopian tubes were slightly reduced in comparison to analyses that included powder use irrespective of the occurrence of tubal ligation or hysterectomy (e.g., ORs among women with intact tubes = 1.23 [95% CI: 0.93–1.64] and 1.44 [95% CI: 0.92–2.24], for all ovarian tumors combined and for borderline tumors, respectively). Associations with any perineal powder used ≥15 years of age were slightly stronger (e.g., ORs among such women = 1.30 [95% CI: 0.99–1.71] and 1.63 [95% CI: 1.07–2.49], for all ovarian tumors combined and for borderline tumors, respectively).

Risk of mucinous borderline tumors was particularly elevated among women who reported any regular use of perineal dusting powder (OR = 1.78, 95% CI: 0.98–3.23), with a lesser risk increase for serous borderline tumors (Table 3). We observed no association of perineal powder use with risk of serous invasive tumors (OR = 1.01, 95% CI: 0.69–1.47), and some suggestion that risk was elevated for the combined group of endometrioid and clear cell invasive tumors (OR = 1.53, 95% CI: 0.91–2.57). Similar to the overall results, we observed no association with measures of extent of the use of perineal dusting powder for any specific histologic subtype. Elevations in risk noted in our overall results among women in the most

recent category of age at or time since first use and in the most recent (1980 or later) calendar period of initiation of powder use were broadly similar across histologic subtypes.

Discussion

A number of case–control studies of ovarian cancer, in addition to ours, have examined the issue of genital powder exposure as a potential risk factor. The validity of all of these studies, including ours, may be influenced by the level of non-response among cases and controls, and by the potential for misclassification (differential and non-differential) of exposure status. The latter derives not just from errors in the recall of the use of genital powder, but from the fact that the presence or concentration of talc can vary from brand to brand and even within one brand of powder over time. Therefore, even when respondents are asked specifically about perineal exposure to powders that contain talc (as in our study), they may be unable to provide accurate information. Reporting of the use of pure cornstarch powder, however, was quite uncommon in this study; if this information is accurate (and this pattern of use extends to other populations), and if the presence, rather than concentration, of talc in dusting powder is the primary determinant of meaningful exposure, then measures of genital powder use of any type may yet serve as a reasonable surrogate for talc exposure.

In support of an inference that genital exposure to powders has the capacity to cause ovarian cancer is the observation of a 30–60% increase in risk across most case–control studies [3]; in this regard, our findings are similar to prior studies. However, a non-causal interpretation may be consistent with the absence of an overall association in the one cohort study of the question [6], along with the absence in most studies (including the current study) of a trend of increasing risk with increasing duration of exposure [3]. However, ovarian talc particle burden has been found not to correlate with the reported number of lifetime applications [10], which (if not reflective of inaccurate reporting) may indicate that duration of the powder use is not relevant when assessing risk associated with differing levels of exposure to talc.

While the increased risk that we observed was largely restricted to borderline tumors, some studies have reported results either similar to [e.g., 11] or different from [e.g., 5, 12] these latter findings. Also, our results add further inconsistency to the results regarding the strength of association of the perineal powder use with histologic subtypes of disease. In particular, we noted no increase in risk of serous invasive disease, in contrast to some [e.g., 4-6] studies—including the single cohort study [6]—that reported the strongest associations with that subtype. Analyses aimed at examining perineal powder during specific time intervals—whether by calendar year, recency of use, or life intervals in which constituents of perineal powder might ascend through the reproductive tract unimpeded by, e.g., closure of the fallopian tubes—either failed to sharpen exposure-disease relationships or yielded results opposite to those that had been observed or hypothesized by others. For example, Wu et al. [5] observed higher risks among women who initiated talc use before 1975, consistent with the hypothesis that products in use before that year were more likely to be carcinogenic owing to contamination with asbestos fibers; in contrast, we noted stronger associations among women who had only used perineal powder during or after 1980.

It is not evident how (or if) additional investigation will be able to resolve the issue of whether perineal exposure to talc predisposes to ovarian malignancy. Further case–control studies will continue to be hindered by the limitations mentioned above. Data from additional cohort studies would be welcome, but without details concerning the composition of the powders used by cohort members—details that many participants may not be able to provide—the results of such studies may similarly be ambiguous in their interpretation.

Acknowledgments

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Table 1

Risk of epithelial ovarian cancer in relation to various sources of genital powder exposure overall and among women with borderline and invasive tumors

	Controls	Borderline tumors			Invasive tumors			All tumors		
	(*n* = 1,313)[a]	(*n* = 217)[a]	OR[b]	95% CI	(*n* = 595)[a]	OR[b]	95% CI	(*n* = 812)[a]	OR[b]	95% CI
Used powder after bathing										
No	1,161	184	1.00	Ref.	515	1.00	Ref.	699	1.00	Ref.
Yes	151	33	1.55	1.02–2.37	79	1.17	0.87–1.58	112	1.27	0.97–1.66
Used powder on sanitary napkins										
No	1,197	201	1.00	Ref.	552	1.00	Ref.	753	1.00	Ref.
Yes	109	16	1.03	0.58–1.84	39	0.75	0.51–1.12	55	0.82	0.58–1.16
Used powder on diaphragm[c]										
No	321	44	1.00	Ref.	116	1.00	Ref.	160	1.00	Ref.
Yes	121	9	0.60	0.27–1.33	37	0.77	0.49–1.21	46	0.72	0.48–1.10
Used vaginal deodorant spray										
No	1,185	194	1.00	Ref.	532	1.00	Ref.	726	1.00	Ref.
Yes	125	23	1.20	0.74–1.95	61	1.14	0.81–1.59	84	1.15	0.85–1.56

[a] Numbers in column may not sum to total due to missing values

[b] Adjusted for age, calendar year of diagnosis/reference date, county of residence, number of full-term births, and duration of hormonal contraception

[c] Restricted to diaphragm users

Table 2

Risk of epithelial ovarian cancer in relation to the use of perineal powder after bathing by duration and timing of use, overall and among women with borderline and invasive tumors

	Controls	Borderline tumors			Invasive tumors			All tumors		
	(*n* = 1,313)[a]	(*n* = 217)[a]	OR[b]	95% CI	(*n* = 595)[a]	OR[b]	95% CI	(*n* = 812)[a]	OR[b]	95% CI
Never used[c]	1,161	184	1.00	Ref.	515	1.0	Ref.	699	1.0	Ref.
Duration of use (years)										
1–9.9	38	9	1.33	0.61–2.87	24	1.42	0.83–2.43	33	1.39	0.85–2.28
10–19.9	35	10	1.97	0.93–4.17	19	1.28	0.71–2.29	29	1.46	0.87–2.45
20–34.9	40	10	1.83	0.88–3.80	20	1.11	0.63–1.95	30	1.28	0.78–2.10
35+	38	4	1.08	0.37–3.15	15	0.86	0.46–1.60	19	0.91	0.51–1.62
Lifetime number of applications										
1–1,599	36	6	1.05	0.42–2.61	20	1.26	0.71–2.25	26	1.21	0.71–2.06
1,600–4,799	37	17	3.11	1.67–5.78	28	1.72	1.03–2.88	45	2.08	1.32–3.27
4,800–9,999	39	6	1.19	0.49–2.92	14	0.78	0.41–1.48	20	0.87	0.50–1.53
10,000+	37	4	0.98	0.34–2.85	14	0.84	0.44–1.59	18	0.87	0.48–1.57
Age at first use (years)[c]										
<15	27	4	0.89	0.30–2.66	8	0.67	0.30–1.53	12	0.74	0.37–1.50
15–<20	36	8	1.46	0.64–3.31	19	1.10	0.61–1.97	27	1.20	0.71–2.03
20–<30	43	12	1.93	0.98–3.80	20	1.04	0.59–1.81	32	1.25	0.77–2.03
30+	45	9	1.68	0.79–3.60	32	1.68	1.04–2.72	41	1.69	1.08–2.64
Age at last use (years)[c]										
<35	33	10	1.54	0.72–3.28	15	0.97	0.51–1.83	25	1.14	0.66–1.97
35–<50	39	15	2.07	1.09–3.93	20	1.15	0.65–2.03	35	1.42	0.88–2.31
50–<60	36	6	1.39	0.56–3.44	19	1.20	0.67–2.15	25	1.25	0.73–2.13
60+	43	2	0.64	0.15–2.74	24	1.30	0.76–2.25	26	1.21	0.72–2.05
Calendar year of first use[c]										
≤1959	39	5	1.47	0.55–3.92	14	0.73	0.38–1.40	19	0.86	0.48–1.53
1960–1969	38	4	0.82	0.28–2.38	20	1.18	0.66–2.09	24	1.10	0.65–1.89
1970–1979	38	11	1.65	0.81–3.37	15	0.91	0.49–1.69	26	1.12	0.66–1.89

	Controls	Borderline tumors			Invasive tumors			All tumors		
	(*n* = 1,313)[a]	(*n* = 217)[a]	OR[b]	95% CI	(*n* = 595)[a]	OR[b]	95% CI	(*n* = 812)[a]	OR[b]	95% CI
1980+	36	33	2.20	1.11–4.34	30	1.97	1.18–3.28	43	2.03	1.28–3.24
Time since first use (years)[c]										
≤25	41	12	1.78	0.89–3.54	30	1.76	1.07–2.89	42	1.77	1.12–2.78
25–<38	41	14	1.98	1.03–3.79	24	1.25	0.73–2.13	38	1.46	0.91–2.32
38–<45	34	3	0.79	0.23–2.69	13	0.88	0.45–1.72	16	0.87	0.47–1.61
45+	35	4	1.30	0.44–3.83	12	0.72	0.36–1.43	16	0.82	0.44–1.52
Time since last use (years)[c]										
Current user	70	12	1.35	0.71–2.59	40	1.28	0.85–1.94	52	1.30	0.89–1.91
≤12	26	9	2.11	0.94–4.77	17	1.59	0.83–3.02	26	1.74	0.98–3.10
13–23	27	7	1.80	0.75–4.34	7	0.55	0.24–1.29	14	0.85	0.44–1.66
24+	28	5	1.22	0.45–3.29	14	1.10	0.56–2.17	19	1.13	0.61–2.08

[a]Numbers in column may not sum to total due to missing values

[b]Adjusted for age, calendar year of diagnosis/reference date, county of residence, number of full-term births, and duration of hormonal contraception

[c]Use defined as regular use after bathing for at least 1 year

Table 3

Risk of histologic types of invasive and borderline epithelial ovarian cancer in relation to various sources of genital powder

	Borderline tumors[c]				Invasive tumors[c]					
	Mucinous (*n* = 89)		Serous (*n* = 117)		Serous (*n* = 335)		Endometrioid/Clear (*n* = 133)		Other nonmucinous (*n* = 104)	
	N	OR[a] (95% CI)	*N*	OR[a] (95% CI)	*N*	OR[a] (95% CI)	*N*	OR[a] (95% CI)	*N*	OR[a] (95% CI)
Used powder after bathing[b]										
No	74	1.0 (Ref.)	100	1.0 (Ref.)	295	1.0 (Ref.)	112	1.0 (Ref.)	87	1.0 (Ref.)
Yes	15	1.78 (0.98–3.23)	17	1.47 (0.84–2.55)	40	1.01 (0.69–1.47)	21	1.53 (0.91–2.57)	17	1.48 (0.85–2.58)
Duration of use[b] (years)										
1–9.9	2	0.71 (0.16–3.10)	7	1.89 (0.80–4.47)	11	1.16 (0.58–2.33)	6	1.42 (0.56–3.57)	6	2.18 (0.88–5.40)
10–19.9	6	3.12 (1.22–7.97)	3	1.10 (0.33–3.70)	11	1.26 (0.62–2.54)	5	1.62 (0.60–4.41)	3	1.16 (0.35–3.92)
20–34.9	5	2.45 (0.92–6.56)	5	1.60 (0.60–4.22)	8	0.76 (0.35–1.66)	5	1.40 (0.52–3.74)	7	2.25 (0.97–5.24)
35+	2	1.26 (0.29–5.51)	2	1.02 (0.24–4.40)	10	0.91 (0.44–1.88)	5	1.85 (0.68–5.05)	0	0.00 (−)
Age at first use (years)[b]										
<15	1	0.56 (0.07–4.27)	3	1.24 (0.36–4.30)	3	0.44 (0.13–1.47)	3	1.20 (0.34–4.24)	2	1.02 (0.23–4.43)
15–<20	5	2.30 (0.84–6.33)	3	1.01 (0.30–3.43)	10	1.01 (0.49–2.08)	7	1.99 (0.83–4.76)	2	0.63 (0.15–2.72)
20–<30	5	2.14 (0.80–5.68)	6	1.69 (0.69–4.15)	10	0.90 (0.44–1.83)	3	0.68 (0.20–2.31)	6	1.82 (0.74–4.47)
30+	4	1.80 (0.61–5.28)	5	1.77 (0.67–4.66)	17	1.45 (0.81–2.59)	8	2.33 (1.03–5.27)	7	2.21 (0.95–5.11)
Calendar year of first use[b]										
≤1959	3	2.08 (0.59–7.30)	2	1.07 (0.25–4.70)	6	0.50 (0.20–1.20)	5	1.78 (0.64–4.95)	3	0.89 (0.26–3.03)
1960–1969	4	2.12 (0.71–6.32)	0	0.00 (−)	12	1.18 (0.60–2.33)	5	1.38 (0.51–3.76)	2	0.72 (0.17–3.08)
1970–1979	3	1.21 (0.36–4.10)	7	1.94 (0.82–4.57)	6	0.66 (0.27–1.60)	5	1.34 (0.50–3.60)	4	1.38 (0.47–4.06)
1980+	5	2.05 (0.76–5.55)	8	2.50 (1.10–5.64)	16	1.84 (1.00–3.40)	6	1.75 (0.70–4.40)	8	3.07 (1.37–6.88)
Time since first use (years)[b]										
≤25	5	1.79 (0.67–4.82)	7	1.93 (0.83–4.52)	16	1.65 (0.90–3.00)	6	1.58 (0.63–3.93)	8	2.73 (1.22–6.07)
26–<38	5	1.89 (0.71–5.06)	8	2.09 (0.93–4.69)	11	1.05 (0.53–2.10)	8	1.80 (0.79–4.08)	5	1.51 (0.57–4.00)
38–<45	3	2.14 (0.61–7.47)	0	0.00 (−)	7	0.75 (0.33–1.75)	4	1.45 (0.48–4.38)	1	0.41 (0.05–3.07)
45+	2	1.46 (0.33–6.49)	2	1.22 (0.28–5.38)	6	0.56 (0.23–1.37)	3	1.19 (0.34–4.19)	3	1.02 (0.30–3.52)

[a] Adjusted for age, calendar year of diagnosis/reference date, county of residence, number of full term births, and duration of hormonal contraception

[b] Use defined as regular use after bathing for at least 1 year

[c] Not included are 6 endometrioid borderline, 5 other borderline, and 23 mucinous invasive tumors

Exhibit 47

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Use of fertility drugs and risk of ovarian cancer: results from a US-based case-control study

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Abstract

Background—Previous studies examining associations between use of fertility drugs and ovarian cancer risk have provided conflicting results. We used data from a large case-control study to determine whether fertility drug use significantly impacts ovarian cancer risk when taking into account parity, gravidity, and cause of infertility.

Methods—Data from the Hormones and Ovarian Cancer Prediction (HOPE) study were used (902 cases, 1802 controls). Medical and reproductive histories were collected via in-person interviews. Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI). Models were adjusted for age, race, education, age at menarche, parity, oral contraceptive use, breastfeeding, talc use, tubal ligation, and family history of breast/ovarian cancer.

Results—Ever use of fertility drugs was not significantly associated with ovarian cancer within the total HOPE population (OR: 0.93, 95%CI: 0.65–1.35) or among women who reported seeking medical attention for infertility (OR: 0.87, 95%CI 0.54–1.40). We did observe a statistically significant increased risk of ovarian cancer for ever use of fertility drugs among women who, despite seeking medical attention for problems getting pregnant, remained nulligravid (OR: 3.13, 95%CI 1.01–9.67).

Conclusions—These results provide further evidence that fertility drug use does not significantly contribute to ovarian cancer risk among the majority of women; however, women

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who despite infertility evaluation and fertility drug use remain nulligravid, may have an elevated risk for ovarian cancer.

Impact—Our results suggest that fertility drug use does not significantly contribute to overall risk of ovarian cancer when adjusting for known confounding factors.

Keywords

ovarian cancer; fertility drugs; infertility; case-control

Introduction

Ovarian cancer is multifactorial and complex in etiology. Lifestyle factors shown to increase the risk of ovarian cancer include low parity (1–4), late onset of menopause (5, 6) and perineal talc use (7–9). Oral contraceptive (OC) use (10–13), breastfeeding (14–16) and tubal ligation (17–19) have been shown to have a protective effect on ovarian cancer risk. Several theories have been proposed to explain the mechanisms by which these factors affect risk of ovarian cancer. The incessant ovulation hypothesis theorizes that the repeated damage and subsequent repair cycles that occur during ovulation on the epithelial surface of the ovary contributes to DNA damage and increases the risk of developing ovarian cancer (20–23). The gonadotropin hypothesis postulates that exposure to high levels of circulating pituitary gonadotropins, which stimulates the ovarian surface epithelium, plays a role in the development of ovarian cancer (24, 25). Both of these theories suggest that the use of fertility drugs, which often contain gonadotropins and stimulate ovulation, may increase the risk of ovarian cancer.

Fertility drug use has increased markedly in the U.S. (26). Based on data from the 2002 National Survey of Family Growth, 12% of the 61.6 million U.S. women between the ages of 16 and 44 sought infertility services. The use of infertility services was more common among older women, women with higher incomes, and women who were childless (27). The utilization of fertility drugs and other infertility services is expected to continue to rise as the percentage of women who postpone attempts to become pregnant until after the age of 35 increases. Stephen *et al.* projected that the number of infertile women will increase to between 5.4–7.7 million in 2025 (28). Despite the growing number of women seeking fertility treatment, the effects of fertility drug use on ovarian cancer risk remain uncertain. Several early studies reported an association between exposure to fertility drugs and the development of ovarian cancer, which spurred concern regarding the safety of these drugs (29–31). Subsequent studies did not provide evidence of an increased risk of ovarian cancer with the use of fertility drugs (32–37). However, concern regarding fertility drug use remains after other studies reported that women who were exposed to fertility drugs for more than 12 cycles were at an increased risk of ovarian cancer (38, 39). Nulliparous women who failed to conceive after treatment have also been reported to have an increased risk of ovarian cancer (29, 35). Finally, several studies have shown that fertility drug use may increase the risk of borderline ovarian tumors (30, 31, 40–43).

The conflicting results from previous studies might be due to the generally small sample sizes and/or inability to control for important reproductive factors known to influence ovarian cancer risk. Establishing the relationship between fertility drug use and ovarian cancer risk is complicated by the fact that infertility itself increases the risk of ovarian cancer (10, 44–46). It is also of particular importance to account for parity because the frequency of nulliparity is high among infertile women and nulliparity has been established as an important ovarian cancer risk factor (24, 47, 48). The increasing use of fertility drugs necessitates the separation of the effects of underlying infertility and other confounding factors from those of fertility drug use. Ours is one of the largest case-control studies of

ovarian cancer conducted to date. Our objective was to contribute to the debate regarding whether fertility drug use significantly impacts ovarian cancer risk when taking into account parity, gravidity, and cause of infertility.

Material and Methods

Study Population and Data Collection

We used data from the Hormones and Ovarian Cancer Prediction (HOPE) study, a population-based case-control study of ovarian cancer described in detail elsewhere (13, 49). Briefly, subjects were residents of a contiguous region comprising Western Pennsylvania, Eastern Ohio, and Western New York State. All cases were histologically confirmed to have primary epithelial ovarian, peritoneal, or fallopian tube cancers diagnosed between 2003 and 2008. Eligible women were at least 25 years old and were within 9 months of initial diagnosis at the time of recruitment. A total of 902 cases were enrolled. Controls, N=1802, were frequency matched to cases (~2:1) by 5-year age group and telephone area code through random-digit dialing. Women who had undergone a bilateral oophorectomy were ineligible. All study participants provided informed consent. The study was approved by the University of Pittsburgh Institutional Review Board and by the human subject committees at each hospital where cases were identified.

Trained interviewers collected questionnaire data that included detailed reproductive, gynecological, and medical histories as well as information regarding lifestyle and family medical history; a reference date of 9 months before the interview date was used for all participants.

Infertility and Fertility Drug Use

All study participants were asked if they had ever sought medical attention for problems becoming pregnant. Women who responded with “yes” to this question were asked whether their partner was tested, they were personally tested, they were both tested, or if neither of them were tested for infertility. They were also presented with a list of infertility causes and asked whether each was found to be a probable cause for their problems becoming pregnant. Women were able to respond “yes,” “no,” or “don’t know” to whether they were diagnosed with a problem involving: partner’s sperm, their ovaries, ovulation, their fallopian tubes, their cervix, cervical mucous, their uterus, scarring of the uterus, menstruation, endometriosis, or some other problem. For the current analyses, we collapsed the cervix and cervical mucous variables into one cervical problem variable. Similarly, we combined the variables for uterus problems and scarring of the uterus. We chose to collapse these variables because the mechanism affecting infertility is similar for both cervical variables as well as both uterine variables. Combining similar causes of infertility resulted in a greater number of exposed women and increased our power to determine whether uterine or cervical causes of infertility were significantly associated with ovarian cancer risk.

All study participants were asked if they had ever used fertility drugs. Women who responded with “yes” to this question were asked the name of the fertility drugs used. The majority of women used clomiphene citrate, which we defined as one group of fertility drugs (“clomiphene”). We pooled follicle stimulating hormone (FSH), human chorionic gonadotropin (hCG), gonadotropin-releasing hormone (GnRH), urofollitropin, and human menopausal gonadotropin (hMG) drugs into one group of fertility drugs, “gonadotropins”, because they utilize the same method of stimulating ovulation. We also created a group for women who had used a combination of gonadotropins and clomiphene citrate (“clomiphene + gonadotropins”). Finally, we grouped together any other fertility drugs, such as progesterone and unknown hormone pills, into an “other” fertility drug group (“other

fertility drug"). Women who reported taking fertility drugs were also asked how many months they took each fertility drug. This information was collected for the first four periods of fertility drug use. We do not have information regarding type of fertility drug or the duration of use for fertility drugs used after the first four time periods of fertility drug use; however, only 9 women reported using fertility drugs for more than four time periods.

Covariates

Based on anthropometric data provided by the participants, we calculated body mass index (BMI) as weight (kg) at reference date divided by height (m) at reference date squared. Family history of ovarian and breast cancers was defined as having at least one reported diagnosis of, respectively, ovarian or breast cancer among a first-degree relative. Hormone replacement therapy (HRT) use was defined as the use of hormones for menopause, to treat osteoporosis, or after hysterectomy/removal of ovaries; any use of estrogen or estrogen plus progesterone among postmenopausal women was also classified as HRT use. Women were classified as postmenopausal if they were 55 years or older, reported natural menopause, had used HRT, or reported no menstrual periods in the 6 months prior to the reference date. Women were considered to be premenopausal if they had never taken HRT and reported having menstrual periods in the 6 months prior to the reference date, and were younger than 55 years old (50). All participants were asked if they had ever been pregnant. Women reporting at least 1 pregnancy were subsequently asked to provide information regarding the outcome of the pregnancy and the duration they breastfed. This information was repeated for up to four pregnancies. Duration of breastfeeding was calculated as the sum of the number of months they breastfed after each of their first four pregnancies. Information regarding pregnancy outcomes, and breastfeeding was not available for later pregnancies; however, women did report their total number of pregnancies and live births. Among women who reported more than four pregnancies, we calculated their average length of breastfeeding for their first four pregnancies, multiplied this average by the number of additional pregnancies resulting in live births, and added this to the total months of reported breastfeeding. Perineal talc use was defined as ever using dusting powder or deodorizing spray on: the genital or rectal areas, on sanitary napkins, on underwear, or on diaphragms or cervical caps.

Statistical Analysis

Associations between ovarian cancer risk and demographic and reproductive factors were evaluated using logistic regression models. These models were used to calculate odds ratios (OR) and corresponding 95% confidence intervals (95% CI), as well as p-trend values for continuous factors.

Backward stepwise regression was used to determine which demographic and reproductive variables should be included as covariates in the regression models used to evaluate the effect of exposure to fertility drugs on ovarian cancer risk. Age was locked into the stepwise model as a continuous variable; a p-value criterion of 0.10 was used to identify additional covariates. The following variables were evaluated for inclusion: race (white, black, other), education (less than high school graduate, high school graduate, post-high school education), site (Pittsburgh, Cleveland, Buffalo), BMI (<25, 25–29.99, ≥30), family history (none, first-degree breast, first-degree ovarian, first-degree ovary and breast), tubal ligation (yes, no, missing), OC use (continuous), number of live births (0, 1, 2, 3, 4, ≥5), breastfeeding (never, < 6, 6 < 12, ≥12 months), age at menarche (continuous), menopausal status (premenopausal, postmenopausal), perineal talc use (ever, never), and HRT use (ever, never). All models are adjusted for the covariates identified through this process with the exception of models in which collinearity occurred between these covariates and the variables of interest (indicated with the results).

Associations between ovarian cancer risk and ever versus never use of fertility drugs and also duration of use, which was evaluated as a continuous variable and as a categorical variable (never, < 6 months, ≥6 months), were evaluated among the total HOPE population and separately among women who reported seeking medical attention for infertility. We chose 6 months as the cutoff for duration of use because this was the median duration of fertility drug use among all women who had taken fertility drugs and using this grouping provided adequate sample size for each group when stratifying for parity and gravidity. Among women who reported seeking medical attention for infertility, we additionally evaluated associations between ovarian cancer risk and year medical attention was sought, who was tested, and underlying cause of infertility using unconditional logistic regression. We also determined whether the relationship between fertility drug use and ovarian cancer risk was modified by year medical attention was sought, age at which medical attention for infertility was sought, cause of infertility, and person tested for infertility problems by creating interaction terms between fertility drug use and these variables and including them in the adjusted model. Finally, we evaluated whether use of specific types of fertility drugs (clomiphene, gonadotropins, clomiphene + gonadotropins, other fertility drugs) was associated with ovarian cancer risk. These analyses were repeated separately for invasive and borderline ovarian tumors; analyses were also repeated using all cases and controls within the HOPE study population.

To examine the impact of parity and gravidity on the association between fertility drug use and ovarian cancer risk, we evaluated ever compared to never use of fertility drugs while stratifying by the following groups of women: parous, nulliparous-gravid, and nulligravid. These analyses were conducted among women who reported seeking medical attention for infertility and repeated using the total HOPE study population.

All significance tests were two-sided; *P* values <0.05 were considered statistically significant. All analyses were conducted using Stata version 12.1 (StataCorp, College Station, TX).

Results

Demographic and reproductive characteristics of the HOPE study population are presented in Table 1. Compared to Caucasians, African Americans had a significantly increased risk of ovarian cancer. High-school graduates and women with post-high school education had a significantly decreased risk of ovarian cancer compared to women with less than a high school education. The following variables were also significantly associated with ovarian cancer risk: age at menarche, OC use, parity, gravidity, duration of breastfeeding, perineal talc use, and tubal ligation. Seeking medical attention for infertility was not significantly associated with ovarian cancer risk (Table 1). Backward stepwise regression yielded a model that included age, race, education, age at menarche, OC use, parity, duration of breastfeeding, perineal talc use, and tubal ligation. First-degree family history of breast/ovarian cancers was associated with a p-value of 0.14 using this method but was nevertheless included in the model because of its known association with ovarian cancer risk.

Table 2 provides medical information for the 445 women who reported seeking medical attention for infertility. No statistically significant association with ovarian cancer was observed for age at which women sought medical attention, year medical attention was initially sought or with person tested for infertility problems. None of the causes of infertility were significantly associated with ovarian cancer risk; however, borderline significant associations were observed for ovulation problems and menstrual problems.

Among the 47 women who reported ovulation problems, 11 had also reported an issue with their menstrual cycles.

Use of fertility drugs was reported by 148 (33%) of the women seeking medical attention for infertility (Table 2). The majority used fertility drugs for less than 12 months (66.7%); mean duration was 11.4 months (range: 1–134 months). Ever use of fertility drugs was not significantly associated with ovarian cancer risk (Table 2) and remained non-significant after additional adjustment for cause of infertility (OR: 0.66, 95%CI: 0.36–1.22), age medical attention was sought (OR: 0.86, 95%CI: 0.53–1.40), year attention was sought (OR: 0.90, 95%CI: 0.58–1.38), and who was tested for infertility problems (no one tested or partner-only tested compared to self tested or partner and self tested, OR: 0.90, 95%CI: 0.54–1.49) (not in table). No significant interactions between fertility drug use and these variables were observed (data not shown). Similar results were observed for duration of fertility drug use (Table 2 and data not shown). Regarding specific types of fertility drugs, the majority of women who ever used fertility drugs reported using only clomiphene citrate (56.1%). None of the drugs evaluated were significantly associated with ovarian cancer risk when looking at ever compared to never use (Table 2) or duration of use (data not shown). Analyses were repeated excluding the 12 cases and controls that reported using unknown or other fertility drugs and the results were unchanged. Additionally, no significant associations between ever use of fertility drugs and ovarian cancer risk were observed when separately assessing borderline (OR: 0.96, 95%CI: 0.31–2.94; adjusted for age, duration of OC use, talc, and age at menarche) and invasive tumors (OR: 0.85, 95%CI: 0.52–1.39; adjusted for all covariates identified by stepwise regression).

Among all 2704 HOPE participants, 152 (5.6%) women reported ever using fertility drugs, this included the 148 women who reported seeking medical attention for infertility and 4 women who had used fertility drugs but had never sought medical attention for fertility issues. All 4 of these latter women were controls; 2 reported taking clomiphene only and 2 reported taking gonadotropins only. Data regarding why these four women reported taking fertility drugs without ever seeking medical attention for infertility were not collected. Ever use of fertility drugs was not significantly associated with ovarian cancer risk in the total HOPE population (OR: 0.93, 95%CI: 0.65–1.35), nor was duration of use (never compared to <6 months of use, OR: 1.05, 95%CI: 0.61–1.80; never compared to ≥6 months of use, OR: 0.82, 95%CI: 0.50–1.34), adjusting for age, race, education, tubal ligation, age of menarche, duration of OC use, number of live births, duration of breastfeeding, perineal talc use, and family history. Adjusting for the same covariates, no significant associations between ovarian cancer risk and ever use of fertility drugs were observed when separately evaluating borderline (OR: 0.64, 95%CI: 0.26–1.55) and invasive tumors (OR: 1.02, 95%CI: 0.69–1.50).

Table 3 presents results of the evaluation of associations between fertility drug use and ovarian cancer risk stratified by parity and gravidity. Among those seeking medical attention for infertility, nulligravid women who used fertility drugs were significantly more likely to develop ovarian cancer than nulligravid women who had never used fertility drugs. However, fertility drug use among parous and nulliparous-gravid women was not significantly associated with ovarian cancer risk among this group of women. Within the total HOPE study population, the association between ovarian cancer risk and ever use of fertility drugs was non-significant among parous and nulliparous-gravid women. Ovarian cancer risk was elevated among nulligravid fertility drug users; however, this was not significant (Table 3).

Discussion

In this large case-control study, we evaluated whether fertility drug use significantly affects ovarian cancer risk when taking into account, parity, gravidity, and cause of infertility. Consistent with results from previous studies, OC use, breastfeeding, and tubal ligation significantly decreased ovarian cancer risk in our study population while nulliparity, and perineal talc use increased risk (19, 24, 36, 40, 51). Ever use of fertility drugs was not significantly associated with ovarian cancer risk within the total HOPE population or among women who reported seeking medical attention for infertility. Risk did not differ significantly according to duration of use or type of fertility drug. However, we did observe a statistically significant increased risk of ovarian cancer for ever use of fertility drugs among women who, despite seeking medical attention for problems getting pregnant, remained nulligravid.

When examining specific causes of infertility among those seeking medical attention for infertility, none of the evaluated causes were significantly associated with ovarian cancer risk. Specifically, we observed no significant association between ovarian cancer and endometriosis even though previous studies have reported an increased risk (40, 52–54). Endometriosis was also not significantly associated with ovarian cancer risk in the total HOPE population (data not shown). The mechanism by which endometriosis may affect ovarian cancer risk is poorly understood; however, several studies have shown that endometriosis-associated tumors are most commonly linked to clear cell and endometrioid tumors (55–58). The small number of women who reported being diagnosed with endometriosis among those who sought medical attention for infertility in addition to the homogeneity of tumor histologic subtypes among these women may have contributed to the null relationship we observed here. Interestingly, we observed a decreased risk of ovarian cancer among women who reported an ovulation problem as their cause of infertility. Although this observation was of borderline significance, it suggests that women who ovulate less frequently throughout their lifetime may have a decreased risk of ovarian cancer and provides further evidence for the incessant ovulation theory.

In a 2004 case-control study, Rossing et al. observed that women whose infertility manifested past the age of 30 were at increased risk of ovarian cancer (36). We found no significant association between ovarian cancer risk and the age at which women sought medical attention for infertility in our population; however, women who sought help between the ages of 35 and 45 did exhibit a non-significant increased risk compared to women who sought help before they were 25. Women who seek treatment for infertility past the age of 30 have a lower likelihood of success compared to women who seek infertility treatments at younger ages (59) and ovarian cancer risk associated with infertility among older women may reflect additional risk associated with low parity among these women.

Although we did not observe any significant associations between fertility drug use and ovarian cancer risk within the total HOPE study population or among the subset of women who reported seeking medical attention for infertility, we did observe, similar to previous reports, a statistically significant increased risk of ovarian cancer associated with ever fertility drug use among nulligravid women who had infertility problems (29, 35, 40). This suggests that women who never became pregnant despite efforts to conceive are at uniquely increased risk of ovarian cancer. This is further supported by the fact that we found no significant association between fertility drug use and ovarian cancer risk among nulliparous women who had at least one pregnancy. Although our results are in line with those from previous studies, it should be noted that the number of nulligravid women who sought medical attention for infertility was relatively small (N=74). Therefore, confirmation of our results by other studies is necessary.

Our finding that fertility drug use does not significantly contribute to ovarian cancer risk among the majority of women is in line with results from other, recent studies (34, 36, 40, 52). Early studies that reported an increased risk of ovarian cancer among fertility drug users included small numbers of ovarian cancer patients exposed to fertility drugs and were unable to adjust for risk factors known to impact ovarian cancer risk (29, 30). We observed no risk difference between borderline and invasive tumors; these results are in agreement with a recent case-control study (60) but disagree with several previous studies (30, 31, 40–43).

The strengths of this study include a large sample size and availability of detailed reproductive and medical histories of women included in the study. The ability to stratify and adjust for factors linked to ovarian cancer risk allowed us to disentangle risk associated with these factors from risk associated with fertility drug use. A limitation of our study is that we were unable to identify women who were infertile but never sought medical attention. This differential misclassification may have attenuated the associations between infertility and ovarian cancer risk. However, our ability to analyze associations between fertility drug use and ovarian cancer risk in a relatively large subset of women who had sought medical attention for infertility greatly improved the comparability of fertility drug users to non-users. Being able to reduce the study population to only these women also limited biases associated with comparing fertility drug users with infertility issues to non-fertility drug users with no history of infertility issues. Our study is also limited by its reliance on self-reported use of fertility drugs; however, the use of a life calendar during interviews may have improved the accuracy of recalling details about fertility drug use. This study includes a greater number of ovarian cancer cases exposed to fertility drugs than previous studies. Despite this, our study had limited power when completing stratified analyses for fertility drug use and ovarian cancer risk, which resulted in small subgroups and subsequently wide confidence intervals.

Our results build upon previous research and provide further evidence that fertility drug use does not significantly contribute to overall risk of ovarian cancer when adjusting for known confounding factors. Our observation that fertility drug use was only significantly associated with increased ovarian cancer risk among nulligravid women who had ever sought medical attention for infertility suggests that a biological mechanism associated with the inability to conceive may impact ovarian cancer risk to a greater extent than fertility medications do.

To conclude, these results are reassuring for women and clinicians embarking on fertility drug usage in the setting of infertility treatment.

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Abbreviations used

CI	confidence intervals
FSH	follicle stimulating hormone
GnRH	gonadotropin-releasing hormone
hCG	human chorionic gonadotropin
hMG	human menopausal gonadotropin
LH	luteinizing hormone

OC	oral contraceptive
OR	odds ratio

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Table 1

Demographic and reproductive characteristics of the total HOPE population.

	Cases (902)		Controls (1802)		OR (95% CI) [a]	p-trend [b]
	N	%	N	%		
Site						
Buffalo	251	27.8	476	26.4	1.0 (ref.)	---
Cleveland	294	32.6	628	34.9	0.89 (0.72, 1.09) [c]	
Pittsburgh	357	39.6	698	38.7	0.97 (0.79, 1.18) [c]	
Age (in years)						
< 30	13	1.4	24	1.3	1.0 (ref.)	0.01
30 < 40	47	5.2	108	6.0	0.80 (0.38, 1.71) [c]	
40 < 50	164	18.2	393	21.8	0.77 (0.38, 1.55) [c]	
50 < 60	276	30.6	569	31.6	0.90 (0.45, 1.79) [c]	
60 < 70	211	23.4	403	22.4	0.97 (0.48, 1.94) [c]	
≥70	191	21.2	305	16.9	1.16 (0.57, 2.33) [c]	
Race						
White	856	94.9	1,758	97.6	1.0 (ref.)	---
Black	35	3.9	29	1.6	2.48 (1.51, 4.08) [c]	
Other	11	1.2	15	0.8	1.51 (0.69, 3.29) [c]	
Education						
Non-high school graduate	83	9.2	82	4.5	1.0 (ref.)	---
High school graduate	303	33.6	535	29.7	0.59 (0.42, 0.83) [d]	
Post-high school	516	57.2	1,185	65.8	0.46 (0.33, 0.64) [d]	
Smoking Status						
Never Smoker	458	50.8	913	50.7	1.0 (ref.)	---
Former Smoker	286	31.7	545	30.2	1.02 (0.84, 1.22)	
Current Smoker	158	17.5	344	19.1	0.86 (0.69, 1.08)	
Body Mass Index (in kg/m^2) [e]						
< 25	300	33.3	671	37.2	1.0 (ref.)	0.08

	Cases (902)		Controls (1802)		OR (95% CI) [a]	p-trend [b]
	N	%	N	%		
25 – 29.99	267	29.6	528	29.3	1.09 (0.89, 1.33)	
≥30	334	37.0	602	33.4	1.18 (0.97, 1.43)	
Family History (1st degree)						
No	715	79.3	1,491	82.7	1.0 (ref.)	---
Breast Cancer Only	147	16.3	255	14.2	1.21 (0.96, 1.51)	
Ovarian Cancer Only	32	3.5	44	2.4	1.51 (0.95, 2.42)	
Breast and Ovarian Cancers	8	0.9	12	0.7	1.21 (0.48, 3.00)	
Age at Menarche (in years)						
<12	182	20.2	444	24.6	1.0 (ref.)	0.22
12	257	28.5	463	25.7	1.38 (1.09, 1.74)	
13	243	26.9	484	26.9	1.26 (0.99, 1.59)	
≥14	220	24.4	411	22.8	1.27 (1.00, 1.62)	
Menopausal Status						
Premenopausal	234	25.9	482	26.8	1.0 (ref.)	---
Postmenopausal	668	74.1	1,320	73.2	0.80 (0.63, 1.03)	
Oral Contraceptive Use (in months) [f]						
Never	367	40.7	531	29.5	1.0 (ref.)	< 0.01
< 6	96	10.6	161	8.9	0.88 (0.65, 1.18)	
6 < 24	135	15.0	282	15.6	0.69 (0.53, 0.89)	
24 < 60	122	13.5	297	16.5	0.61 (0.47, 0.79)	
60 < 120	123	13.6	299	16.6	0.63 (0.48, 0.82)	
≥ 120	58	6.4	232	12.9	0.37 (0.27, 0.52)	
Hormone Replacement Therapy Use						
Never	543	60.2	1039	57.7	1.0 (ref.)	---
Ever	359	39.8	763	42.3	0.87 (0.73, 1.03)	
Number of Pregnancies						
0	167	18.5	167	9.3	1.0 (ref.)	< 0.01
1	114	12.6	188	10.4	0.57 (0.41, 0.78)	
2	216	24.0	458	25.4	0.44 (0.33, 0.58)	
3	167	18.5	426	23.6	0.36 (0.27, 0.47)	

	Cases (902)		Controls (1802)		OR (95% CI) [a]	p-trend [b]
	N	%	N	%		
4	112	12.4	284	15.8	0.34 (0.25, 0.46)	
≥5	126	14.0	279	15.5	0.34 (0.25, 0.47)	
Number of Live Births						
0	213	23.6	230	12.8	1.0 (ref.)	< 0.01
1	117	13.0	228	12.7	0.51 (0.38, 0.68)	
2	263	29.2	593	32.9	0.45 (0.35, 0.57)	
3	170	18.8	418	23.2	0.39 (0.30, 0.51)	
4	73	8.1	190	10.5	0.32 (0.23, 0.45)	
≥5	66	7.3	143	7.9	0.32 (0.22, 0.47)	
Duration of Breastfeeding (in months)						
Never	610	67.6	928	51.5	1.0 (ref.)	< 0.01
< 6	117	13.0	296	16.4	0.60 (0.47, 0.76)	
6 < 12	66	7.3	199	11.0	0.54 (0.40, 0.72)	
≥ 12	109	12.1	379	21.0	0.46 (0.36, 0.59)	
Perineal Talc Use						
No	653	72.4	1426	79.1	1.0 (ref.)	---
Yes	249	27.6	376	20.9	1.40 (1.16, 1.69)	
Tubal Ligation						
No	666	73.8	1162	64.5	1.0 (ref.)	---
Yes	201	22.3	616	34.2	0.55 (0.46, 0.67)	
Unknown	35	3.9	24	1.3	2.66 (1.57, 4.53)	
Sought Medical Attention for Infertility						
Never	747	82.8	1512	83.9	1.0 (ref.)	---
Ever	155	17.2	290	16.1	1.15 (0.93, 1.43)	

[a] Odds ratios and corresponding confidence intervals are adjusted for age (continuous), race (white, black, other), and education (non-high school graduate, high school graduate, post high-school), unless otherwise noted.

[b] P-trend values were obtained from logistic regression models by using continuous versions of these factors; all models were adjusted for age, race, and education with the exception of age, which was unadjusted.

[c] Unadjusted.

[d] Adjusted for age and race.

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[e] 1 case and 1 control were missing weight information.

[f] 1 case was missing oral contraceptive use information.

Table 2

Medical information, infertility causes, and ovarian cancer risk among HOPE participants seeking medical attention for infertility (N=445).

	Cases (155)		Controls (290)		OR (95% CI) [a]
	N	%	N	%	
Year Medical Attention was Sought					
≤1970	55	35.5	97	33.5	1.0 (ref.)
1970≤1980	39	25.2	76	26.2	1.13 (0.55, 2.31) [b]
1980≤1990	31	20.0	74	25.5	0.77 (0.31, 1.91) [b]
After 1990	30	19.3	43	14.8	1.09 (0.34, 3.47) [b]
Age at Which Medical Attention was Sought (in years)					
< 25	47	30.3	86	29.7	1.0 (ref.)
25 < 30	52	33.5	110	37.9	0.94 (0.55, 1.61) [b]
30 < 35	35	22.6	68	23.4	0.89 (0.48, 1.66) [b]
35 < 40	17	11.0	18	6.2	2.00 (0.84, 4.75) [b]
≥40	4	2.6	8	2.8	0.84 (0.21, 3.37) [b]
Fertility Testing Done					
None	20	12.9	50	17.2	1.0 (ref.)
Partner	12	7.7	17	5.9	1.41 (0.53, 3.75)
Self	55	35.5	84	29.0	1.32 (0.66, 2.67)
Both	68	43.9	139	47.9	0.92 (0.47, 1.81)
Fertility Drug Use					
Never	105	67.7	192	66.2	1.0 (ref.)
Ever	50	32.3	98	33.8	0.87 (0.54, 1.40)
Type of Fertility Drug					
Never	105	67.7	192	66.2	1.0 (ref.)
Clomiphene Only	28	18.1	55	19.0	0.87 (0.49, 1.56) [b]
Gonadotropin Only	7	4.5	20	6.9	0.51 (0.20, 1.32) [b]
Gonadotropin + Clomiphene Only	9	5.8	17	5.8	0.94 (0.37, 2.42) [b]
Other Only [c]	6	3.9	6	2.1	1.87 (0.53, 6.65) [b]

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	Cases (155)		Controls (290)		OR (95% CI) [a]
	N	%	N	%	
Duration of Fertility Drug Use (in months) [d]					
Never	105	67.7	192	66.2	1.0 (ref.)
< 6	22	14.2	41	14.1	0.92 (0.48, 1.74) [b]
≥6	27	17.4	57	19.7	0.75 (0.42, 1.34) b
Low Sperm Count [e]					
No	130	83.9	229	79.0	1.0 (ref.)
Yes	25	16.1	55	19.0	0.68 (0.39, 1.18) [b]
Problems with ovaries (cysts) [e]					
No	141	91.0	264	91.0	1.0 (ref.)
Yes	14	9.0	21	7.2	1.32 (0.61, 2.84) [b]
Ovulation Problems [e]					
No	144	92.9	248	85.5	1.0 (ref.)
Yes	11	7.1	36	12.4	0.51 (0.24, 1.09) [b]
Tubal Problems [e]					
No	137	88.4	245	83.5	1.0 (ref.)
Yes	18	11.6	40	13.8	0.62 (0.33, 1.18) [b]
Uterine Problems [e]					
No	147	94.8	274	94.5	1.0 (ref.)
Yes	8	5.2	11	3.8	1.04 (0.38, 2.83) [b]
Menstrual Problems [e]					
No	146	94.2	254	87.6	1.0 (ref.)
Yes	9	5.8	30	10.3	0.48 (0.20, 1.11) [b]
Endometriosis [e]					
No	141	91.0	259	89.3	1.0 (ref.)
Yes	13	8.4	25	8.6	0.75 (0.35, 1.59) [b]
Cervical Problems [e]					
No	152	98.1	277	95.5	1.0 (ref.)

	Cases (155)		Controls (290)		OR (95% CI) [a]
	N	%	N	%	
Yes	3	1.9	8	2.8	0.53 (0.11, 2.59) [b]
Other Diagnosis [e]					
No	126	81.3	240	82.8	1.0 (ref.)
Yes	29	18.7	46	15.9	1.56 (0.87, 2.79) [b]

[a] ORs and corresponding 95% CIs are adjusted for age, race, education, tubal ligation, age of menarche, duration of oral contraceptive use, number of live births, duration of breastfeeding, perineal talc use, and family history of breast/ovary cancers.

[b] Due to collinearity, family history of breast/ovarian cancer was omitted from the adjusted logistic regression model. These ORs and corresponding 95% CIs are adjusted for all other variables listed in [a].

[c] Includes the following fertility drugs: roloxifene, danazol, unknown hormone pills, bromocriptine, progesterone, and metformin.

[d] Duration of fertility drug use was missing for one case and was therefore not included in the logistic regression model; percentages correspond to the entire population of women who sought medical attention for problems getting pregnant.

[e] These variables exclude women who responded “don’t know” when asked if they were diagnosed with a particular infertility problem and these women were also not included in logistic regression models. Percentages correspond to the entire population of women who sought medical attention for problems getting pregnant.

Table 3

Ovarian cancer risk according to parity, gravidity, and fertility drug use in total HOPE population and separately among HOPE participants that sought medical attention for infertility.

Parity	Gravidity	Women Who Sought Medical Attention for Infertility				Total HOPE Population			
		Fertility Drug Use	Cases (N=155) N(%)	Controls (N=290)	OR (95% CI)	Fertility Drug Use	Cases (N=902)	Controls (N=1802)	OR (95% CI)
Parous		No	80 (51.6)	156 (53.8)	1.0 (ref.)	No	666 (73.8)	1493 (82.8)	1.0 (ref.)
		Yes	23 (14.8)	75 (25.9)	0.57 (0.31, 1.05) [a]	Yes	23 (2.6)	79 (4.4)	0.72 (0.44, 1.19) [a]
Nulliparous	Ever Pregnant	No	8 (5.2)	9 (3.1)	1.0 (ref.)	No	37 (4.1)	52 (2.9)	1.0 (ref.)
		Yes	9 (5.8)	11 (3.8)	0.47 (0.09, 2.53) [b]	Yes	9 (1.0)	11 (0.6)	0.77 (0.26, 2.25) [d]
Nulliparous	Never Pregnant	No	17 (11.0)	27 (9.3)	1.0 (ref.)	No	149 (16.5)	155 (8.6)	1.0 (ref.)
		Yes	18(11.6)	12 (4.1)	3.13 (1.01, 9.67) [c]	Yes	18 (2.0)	12 (0.7)	1.52 (0.68, 3.41) [e]

[a] Adjusted for: age, age of menarche, duration of OC use, perineal talc use, education, family history of breast/ovarian cancers, tubal ligation, race, duration of breastfeeding, and number of live births.

[b] Adjusted for: age, age of menarche, duration of OC use, and perineal talc use.

[c] Adjusted for: age, age of menarche, duration of OC use, perineal talc use, education, and family history of breast/ovarian cancers.

[d] Adjusted for: age, age of menarche, duration of OC use, perineal talc use, education, family history of breast/ovarian cancers, and tubal ligation.

[e] Adjusted for: age, age at menarche, duration of OC use, perineal talc use, education, and tubal ligation.

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Exhibit 48

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Research Article

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African Americans and Hispanics Remain at Lower Risk of Ovarian Cancer Than Non-Hispanic Whites after Considering Nongenetic Risk Factors and Oophorectomy Rates

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Abstract

Background: Risk factors for invasive epithelial ovarian cancer (IEOC) among Hispanics and African Americans are understudied despite notable differences in incidence relative to non-Hispanic whites.

Methods: We used multivariate logistic regression to examine parity, oral contraceptive use, tubal ligation, endometriosis, family history of ovarian cancer, and talc use and risk of IEOC among Hispanics (308 cases and 380 controls), African Americans (128 cases and 143 controls), and non-Hispanic whites (1,265 cases and 1,868 controls) using four case–control studies we conducted in Los Angeles County. We expressed each of these factors in the form of increasing risk and calculated population attributable risk percentage (PAR%) estimates for the six risk factors separately and jointly in the three groups.

Results: The risk associations with these six well-accepted factors were comparable in the three groups. The significant racial/ethnic differences in the prevalence of these factors and differences in their oophorectomy rates explained 31% of the lower incidence in African Americans compared with non-Hispanic whites, but only 13% of the lower incidence in Hispanics. The PAR%s ranged from 27.5% to 31.0% for no tubal ligation, 15.9% to 22.2% for not using oral contraceptives, and 12.2% to 15.1% for using talc in the three groups.

Conclusions: All six risk factors are comparably important in the three groups. Differences in the prevalence of these factors and their oophorectomy rates explained approximately one third of the difference in incidence between African Americans and non-Hispanic whites.

Impact: Devising strategies to lessen the burden of IEOC will be applicable to all three racial/ethnic groups. *Cancer Epidemiol Biomarkers Prev; 24(7); 1094–100. ©2015 AACR.*

Introduction

In the United States in the period 2000 to 2009, the annual age-adjusted incidence rate of invasive epithelial ovarian cancer (IEOC) was highest in non-Hispanic whites (14.3/100,000), intermediate in Hispanics (12.1/100,000; 15% lower than the rate in non-Hispanic whites) and lowest in African Americans (10.2/100,000; 29% lower than the rate in non-Hispanic whites; ref. 1). Epidemiologic studies of ovarian cancer risk have focused primarily on non-Hispanic white women; reasons for the racial/ethnic differences in incidence are not well understood.

A number of risk factors—first-degree family history of ovarian cancer, endometriosis, and use of talc—and protective factors—parity, use of oral contraceptives, and tubal ligation—have been unequivocally associated with ovarian cancer in non-Hispanic whites. There is virtually no information on ovarian cancer risk factors in Hispanics. A small number of Hispanic cases ($n = 42$) were included in an ovarian cancer case–control study conducted in the Central Valley of California, but only results on talc use were reported separately in Hispanics (35.7% in cases vs. 26.9% in controls; ref. 2). A hospital-based case–control study in Mexico compared risk factors between 84 ovarian cancer cases and control women selected from an outpatient clinic (3): Parity and use of oral contraceptives were significantly inversely associated with risk but information on other factors has not been presented.

Risk factors for ovarian cancer among African Americans have been examined in three reports (4–6). The Collaborative Analysis of U.S. Case–Control Studies of Ovarian Cancer included seven studies with a total of 110 ovarian cancers (72 invasive, 35 borderline, and 3 unknown) in African-American women (4). Ness and colleagues (5) reported on risk of ovarian cancer among 84 African-American women with invasive or borderline cancers (numbers of each not specified) from their Delaware Valley case–control study. More recently, Moorman and colleagues (6) reported results from 111 African Americans with invasive ovarian cancer from their North Carolina ovarian cancer case–control study. Reduced risk from increased parity and oral contraceptive use were found in all three studies. Tubal ligation was found to be significantly inversely associated with risk in both of the studies that reported on this factor (5, 6). The results regarding family history are unclear. John and colleagues (4) did not report on family history. Ness and colleagues found that a family history of ovarian cancer was inversely associated with risk in African

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Americans, but this was based on sparse numbers (1.2% of cases vs. 2.0% of controls), a finding contrary to the strong increased risk found in non-Hispanic whites (4.6% of cases vs. 1.9% of controls; ref. 5). Family history of ovarian cancer was not reported in the North Carolina study, but family history of breast or ovarian cancer was a significant risk factor for African Americans (6).

The literature on causes of IEOC in Hispanics and African Americans is, therefore, very limited and it remains unclear to what extent the differences in the prevalence of ovarian cancer risk factors explain the differences in incidence between these three racial/ethnic groups. During the period 1992 to 2008, we conducted four IEOC case–control studies in Los Angeles County designed to elucidate risk factors for the disease and to evaluate differences in risk across non-Hispanic whites, Hispanics, and African Americans.

Materials and Methods

The results presented here are based on pooling the questionnaire data from these four studies, which used identical data collection methods as regards the factors discussed here; comprehensive details of these methods have been published (7–9). These studies were approved by the University of Southern California Institutional Review Board, and written informed consent was obtained from each patient and control before her interview.

Case ascertainment

For all studies, newly diagnosed histologically confirmed IEOC cases were identified from the USC Cancer Surveillance Program, which is the Los Angeles County SEER Program. Eligible patients were female residents of Los Angeles County of self-reported non-Hispanic white, Hispanic, or African-American race/ethnicity. Cases were eligible for inclusion in the study if they were between 18 and 74 years of age at diagnosis (up to age 79 for cases diagnosed between 2003 and 2008). A total of 3,370 patients met the study criteria (2,580 non-Hispanic whites, 506 Hispanics, 284 African Americans). Overall, 15.7% of patients (17.2% non-Hispanic whites, 8.5% Hispanics, and 15.5% African Americans) declined to be interviewed, 16.9% had died or were too ill to be interviewed (17.8% non-Hispanic whites, 12.1% Hispanics, and 17.6% African Americans), and 11.4% could not be located or had moved out of Los Angeles County (10.2% non-Hispanic whites, 14.0% Hispanics, and 17.6% African Americans). We were thus able to carry out in-person interviews with 1,886 patients (1,415 non-Hispanic whites, 331 Hispanics, and 140 African Americans), representing 63.2% participation rate of the patients approached (61.1% non-Hispanic whites, 76.1% Hispanics, and 59.8% African Americans). The response rate was higher for patients diagnosed with localized cancer (69%) compared with those with more advanced stage at diagnosis (61%). Response rates were highest for those diagnosed under age 60 (70%), intermediate for those ages 60 to 69 (59%), and lowest for those ages 70+ (47%) at diagnosis. In this analysis, we excluded 185 patients who had a previous cancer (excluding nonmelanoma skin cancer) or had prior bilateral oophorectomy and the final analysis was based on 1,701 patients (1,265 non-Hispanic whites, 308 Hispanics, and 128 African Americans).

Control ascertainment

Controls were residents of Los Angeles County with at least one intact ovary identified using a well-tested neighborhood control selection algorithm (8–10). Neighborhood controls were individually matched to cases on race/ethnicity and year of birth (±5 years); they represented essentially all the controls interviewed. In one study, selection of controls for cases >65 years of age was augmented, if necessary, by using lists of female residents of Los Angeles County provided by the Health Care Financing Administration, matched to the case on zip code, race/ethnicity, and year of birth closest to the case's year of birth (8). Overall, 70% of the non-Hispanic white, Hispanic, and African-American controls interviewed were the first identified control.

Data collection

In-person interviews were conducted using standardized questionnaires that included the use of a life calendar. The core questions on the risk factors presented here were identical in the four studies. The questionnaire covered events up to 12 months before a case's diagnosis date and a similar reference date for the controls.

The demographic, lifestyle, and medical history variables considered in this analysis include race/ethnicity (African American, Hispanic, and non-Hispanic white), age at diagnosis, parity, oral contraceptive use, tubal ligation, self-reported physician-diagnosed endometriosis, first-degree family history of ovarian cancer, and genital talc use.

Statistical analysis

We used standard statistical methods, including multivariate logistic regression, using the statistical package programs STATA 12 (StataCorp) and SAS 9.2 (SAS Institute Inc.). Although the studies were designed as matched case–control studies, at the termination of the particular studies, some cases had not been matched to a control and there were some controls whose cases had to be excluded after they completed the interview, because they were ineligible for the current analysis (e.g., not IEOC or did not live in Los Angeles County at the time of diagnosis). In this report, we have used all interviewed cases and controls by adopting a stratified multivariate logistic regression analysis approach with joint stratification for the three race/ethnicity groups, age group (<30, 5-year age groups to age 79), interviewer, and study. Analysis focused on the following factors: nulliparity (yes/no), oral contraceptive use (yes/no; no included never and <1 year of use), tubal ligation (yes/no), history of endometriosis (yes/no), family history of ovarian cancer (mother or sister; yes/no), and history of genital talc use (yes/no; no included never and <1 year of use). The logistic regression analysis also adjusted for menopausal status [premenopausal, natural menopause age ≤49, natural menopause age 50–54, natural menopause ≥55, surgical menopause (simple hysterectomy only) age ≤49, surgical menopause ≥50, other], age at menarche (≤11, 12, 13, ≥14), hormone therapy use (none, former or current estrogen + progestin, former or current estrogen alone), body mass index (BMI; kg/m^2; ≤22, >22–24, >24–28, >28), family income (≤40,000, >40,000 to ≤64,000, >64,000 to ≤100,000, >100,000, do not know) and education (high school or less, some college, college or higher). ORs—and corresponding 95% confidence intervals (CI)—were calculated as estimates of the relative risks (RR). All statistical significance values (*P* values) quoted are two-sided.

Population attributable risk percentages (PAR%s), defined as the percentages of disease in the population that are attributable to a given risk factor (or set of risk factors), were calculated using the method of Bruzzi and colleagues (11). These authors showed that PAR%s could be calculated from a case–control study using

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the estimated RRs applied to the cases only. This approach is of particular value to our analysis as it only requires the cases to be a representative sample from the population at risk. This method uses the individual data on each case to calculate the expected fraction of the cases that would not have occurred if the risk factors being considered were at their baseline values, and this fraction was then used to calculate the PAR%. For a single risk factor, the confidence limit for the PAR% was obtained by repeating the calculation using the lower (and upper) confidence bound of the OR for the particular factor in this calculation. For multiple risk factors, the confidence bounds for the PAR% were obtained by simulation: drawing repeated random samples from the mean and covariance matrix of the log ORs from the logistic regression fit and calculating a PAR% from that sample—the 95% CI bounds were taken as the 2.5% and 97.5% values from the repeated samples. In our simulation analyses, we used 5,000 repeats.

Published incidence rates for IEOC make no adjustment for the number of women who have had their ovaries (and fallopian tubes) removed. Writing h for the proportion of women who have had a hysterectomy and t for the proportion of hysterectomies that include removal of the ovaries (oophorectomy), an incidence rate r is approximately adjusted (not accounting for age at oophorectomy) for the oophorectomy rate as follows:

$$r_{\text{adj-ooph}} = r/(1 - h \times t) \quad \text{(A)}$$

If a population incidence rate (or an oophorectomy adjusted incidence rate) r is associated with a PAR% p for a single risk factor (or a group of risk factors) then the expected incidence rate if the population was at the baseline risk of the risk factor is:

$$r_{\text{adj-PAR\%}} = r \times (1 - p/100) \quad \text{(B)}$$

Results

This analysis was based on 1,701 women diagnosed with IEOC (1,265 non-Hispanic whites, 308 Hispanics, and 128 African Americans) and 2,391 control women (1,868 non-Hispanic whites, 380 Hispanics, and 143 African Americans). The distribution of IEOC by histology, stage at diagnosis and differentiation did not differ significantly between the three groups (Table 1). The majority of IEOC in the three racial/ethnic groups was of serous cell type, distant stage at diagnosis, and poorly differentiated.

The prevalence of the risk factors, including the average number of births, duration of oral contraceptive use, and duration of talc use in the three groups of controls and cases, are shown in Table 2. All six factors are presented in the manner of being associated with increasing risk; that is, the factors that are inversely associated with risk are presented in the form of their absence being a risk factor, for example, the decreased risk in parous women is presented as a risk in nulliparous women. This was done to allow the presentation of PAR%s in a standard fashion.

With the exception of family history of ovarian cancer, the prevalence of the other risk factors differed significantly between the three racial/ethnic groups of control women (Table 2, top). The prevalence of no tubal ligation was 69.2% in African-American, 73.7% in Hispanic, and 85.9% in non-Hispanic white control women ($P_{2df} < 0.0001$). Nulliparity and history of endometriosis was highest in non-Hispanic whites, intermediate in African Americans, and lowest in Hispanics (23.7%, 16.8%, and 13.7% for nulliparity, $P_{2df} < 0.001$; 7.5%, 5.6%, and 3.4% for endometriosis, $P_{2df} = 0.008$). No oral contraceptive use (no/<1 year) was highest in Hispanics (54.7%), followed by African Americans (47.6%), and lowest in non-Hispanic whites (41.5%; $P_{2df} < 0.001$). Talc use was more common in African-American women (44.1%) than in non-Hispanic whites (30.4%) or Hispanics (28.9%; $P_{2df} = 0.001$). Similar patterns of differences in these risk factors between the three racial/ethnic groups of IEOC patients were found (Table 2, bottom).

As expected, each of the six risk factors had statistically significant independent effects on risk in non-Hispanic whites. Risk patterns in Hispanics paralleled those in non-Hispanic whites (Table 3), although the elevated risks with endometriosis and family history of ovarian cancer did not achieve statistical significance. In African Americans, family history of ovarian cancer was associated with a more than 7-fold increased risk, but the CI was wide (OR, 7.84; 95% CI, 1.66–37.0). The associations with parity, oral contraceptive use, tubal ligation, endometriosis, and talc use in African Americans are all in agreement with the risks found in non-Hispanic whites, although none were statistically significant.

Table 1. Tumor characteristics of invasive ovarian cancer in non-Hispanic whites, Hispanics, and African Americans: Los Angeles County Ovarian Cancer Study

	Non-Hispanic whites $N = 1,265$	Hispanics $N = 308$	African Americans $N = 128$
Age, y			
<30	12 (0.9%)	5 (1.6%)	1 (0.8%)
30–34	14 (1.1%)	11 (3.6%)	2 (1.6%)
35–39	33 (2.6%)	10 (3.2%)	3 (2.3%)
40–44	58 (4.6%)	31 (10.1%)	13 (10.2%)
45–49	144 (11.4%)	36 (11.7%)	17 (13.3%)
50–54	194 (15.3%)	60 (19.5%)	25 (19.5%)
55–59	186 (14.7%)	46 (14.9%)	18 (14.1%)
60–64	193 (15.3%)	43 (14.0%)	24 (18.8%)
65–69	179 (14.2%)	29 (9.4%)	15 (11.7%)
70–74	160 (12.6%)	23 (7.5%)	8 (6.3%)
75–79	92 (7.3%)	14 (4.5%)	2 (1.6%)
Histology			
Serous	721 (57.0%)	179 (58.1%)	71 (55.5%)
Mucinous	85 (6.7%)	26 (8.4%)	12 (9.4%)
Endometrioid	153 (12.1%)	34 (11.0%)	14 (10.9%)
Clear cell	75 (5.9%)	14 (4.5%)	4 (3.1%)
Epithelial	40 (3.2%)	13 (4.2%)	2 (1.6.%)
Undifferentiated/poorly	53 (4.2%)	12 (3.9%)	10 (7.8%)
Other	131 (10.4%)	28 (9.1%)	14 (10.9%)
Not known	7 (0.6%)	2 (0.6%)	1 (0.8%)
P_{3df}[a,b]		0.54	0.40
Stage			
Localized	216 (17.1%)	58 (18.8%)	30 (23.4%)
Regional	170 (13.4%)	49 (15.9%)	12 (9.4%)
Distant	853 (67.4%)	197 (64.0%)	83 (64.8%)
Not known	26 (2.1%)	4 (1.3%)	3 (2.3%)
P_{2df}[a,c]		0.38	0.12
Differentiation			
Well	119 (9.4%)	29 (9.4%)	9 (7.0%)
Moderately well	235 (18.6%)	53 (17.2%)	28 (21.9%)
Poorly	502 (39.7%)	119 (38.6%)	46 (35.9%)
Undifferentiated	170 (13.4%)	33 (10.7%)	16 (12.5%)
Not known	239 (18.9%)	74 (24.0%)	29 (22.7%)
P_{3df}[a,b]		0.81	0.63

[a]*P* value comparing non-Hispanic whites with each of the other two groups separately.
[b]*P* value based on cases of serous, mucinous, endometrioid, and clear-cell histology only.
[c]*P* value excluding cases with no known histology or stage of cancer at diagnosis.

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Table 2. Prevalence of risk factors in non-Hispanic white, Hispanic, and African-American control women (top) and ovarian cancer cases (bottom)

Factors	Non-Hispanic whites	Hispanics	African Americans	P1[b]	P2[c]	P3[d]
Controls[a]						
Nulliparous (%)	23.7%	13.7%	16.8%	<0.001	0.076	0.45
Mean # births among parous (SD)	2.5 (1.3)	3.0 (1.7)	2.7 (1.5)	<0.001	0.03	0.15
Oral contraceptive use (no/<1 year; %)	41.5%	54.7%	47.6%	<0.001	0.19	0.17
Mean # months of OC use among users (SD)	95.9 (74.9)	81.0 (67.0)	93.1 (74.2)	0.014	0.75	0.21
No tubal ligation (%)	85.9%	73.7%	69.2%	<0.001	<0.001	0.36
Endometriosis (%)	7.5%	3.4%	5.6%	0.006	0.50	0.38
Family history of ovarian cancer (%)	2.5%	3.4%	2.8%	0.37	0.98	0.93
Talc use ≥1 year (%)	30.4%	28.9%	44.1%	0.61	0.0001	0.002
Mean # years of talc use among users (SD)	23.9 (17.4)	21.3 (16.7)	22.9 (17.0)	0.15	0.67	0.55
Cases[e]						
Nulliparous (%)	27.8%	17.9%	16.4%	<0.001	0.007	0.82
Mean # births among parous (SD)	2.5 (1.2)	3.1 (1.7)	2.8 (1.6)	<0.001	0.003	0.24
Oral contraceptive use (no/< 1 year; %)	57.4%	69.8%	50.0%	<0.001	0.13	<0.001
Mean # months of OC use among users (SD)	73.4 (61.1)	59.8 (53.1)	75.7 (66.7)	0.044	0.75	0.10
No tubal ligation (%)	90.6%	83.8%	80.5%	<0.001	<0.001	0.49
Endometriosis (%)	11.1%	5.5%	9.4%	0.005	0.66	0.21
Family history of ovarian cancer (%)	5.1%	4.9%	7.0%	0.96	0.48	0.50
Talc use ≥1 year (%)	41.2%	38.6%	47.7%	0.45	0.19	0.10
Mean # years of talc use among users (SD)	27.5 (18.4)	21.6 (16.9)	26.6 (18.2)	0.001	0.71	0.069

[a]Controls included: 1,868 non-Hispanic whites, 380 Hispanics, and 143 African Americans.
[b]P_{1df} for differences between non-Hispanic whites and Hispanic controls (top)/P_{1df} for differences between non-Hispanic whites and Hispanic cases (bottom).
[c]P_{1df} for differences between non-Hispanic whites and African American controls (top)/P_{1df} for differences between non-Hispanic whites and African American cases (bottom).
[d]P_{1df} for differences between Hispanic and African American controls (top)/P_{1df} for differences between Hispanic and African American cases (bottom).
[e]Cases included: 1,265 non-Hispanic whites, 308 Hispanics, and 128 African Americans.

The adjusted ORs for the three racial/ethnic groups combined are also shown in Table 3.

The first three columns of Table 4 show that these six factors together accounted for 57.9% of IEOCs in non-Hispanic whites compared with 56.1% in Hispanics and 53.8% in African Americans based on the race/ethnicity-adjusted OR estimates shown in Table 3 (last column). The PAR% due to "no tubal ligation" was large in all three racial/ethnic groups, ranging from 27.5% to 31.0%, followed by "no oral contraceptive use" (ranging from 15.9% to 22.2%), and talc use (ranging from 12.2% to 15.1%). The PAR% for nulliparity was 8.9% in non-Hispanic whites, but lower in Hispanics (5.7%) and African Americans (5.5%). The PAR%s for endometriosis (ranging from 2.0% to 4.0%) and family history of ovarian cancer (ranging from 2.7% to 3.9%) were more modest. The large "no tubal ligation" PAR% is due to relatively high prevalence in the IEOC patients (Table 2, bottom);

Table 3. Mutually adjusted ORs[a] for invasive ovarian cancer in Los Angeles County non-Hispanic whites, Hispanics, and African Americans

	Non-Hispanic whites (1,265/1,868)		Hispanics (308/380)		African Americans (128/143)		All (1701/2391)	
	ca/co	OR (95% CI)	ca/co	OR (95% CI)	ca/co	OR (95% CI)	ca/co	OR (95% CI)
Live-births								
Yes	913/1,426	1.00	253/328	1.00	107/119	1.00	1,273/1,873	1.00
No	352/442	1.43 (1.19–1.73)	55/52	2.22 (1.28–3.84)	21/24	1.42 (0.54–3.75)	428/518	1.47 (1.24–1.75)
Per birth		0.70 (0.58–0.84)		0.45 (0.26–0.78)		0.70 (0.27–1.86)		0.68 (0.57–0.81)
Oral contraceptive (OC)								
Yes	539/1,092	1.00	93/172	1.00	64/75	1.00	696/1,339	1.00
None/<1 year	726/776	1.55 (1.31–1.84)	215/208	1.29 (0.87–1.92)	64/68	1.30 (0.64–2.63)	1,005/1,052	1.47 (1.26–1.70)
Per 5 years OC		0.64 (0.54–0.76)		0.77 (0.52–1.15)		0.77 (0.38–1.55)		0.68 (0.59–0.79)
Tubal ligation								
Yes	119/263	1.00	50/100	1.00	25/44	1.00	194/407	1.00
No	1,146/1,605	1.41 (1.10–1.81)	258/280	1.71 (1.07–2.74)	103/99	1.65 (0.73–3.74)	1,507/1,984	1.52 (1.23–1.87)
Endometriosis								
No	1,125/1,728	1.00	291/367	1.00	116/135	1.00	1,532/2,230	1.00
Yes	140/140	1.51 (1.15–1.98)	17/13	2.21 (0.89–5.48)	12/8	1.74 (0.45–6.74)	169/161	1.56 (1.21–2.00)
First-degree family history of ovarian cancer								
No	1,200/1,822	1.00	293/367	1.00	119/139	1.00	1,612/2,328	1.00
Yes	65/46	2.12 (1.40–3.21)	15/13	2.38 (0.94–6.01)	9/4	7.84 (1.66–37.0)	89/63	2.26 (1.58–3.25)
Genital talc use								
None/<1 year	744/1,300	1.00	189/270	1.00	67/80	1.00	1,000/1,650	1.00
Yes	521/568	1.41 (1.21–1.67)	119/110	1.77 (1.20–2.62)	61/63	1.56 (0.80–3.04)	701/741	1.46 (1.27–1.69)
Per 5 years talc		1.14 (1.08–1.21)		1.18 (1.02–1.36)		1.15 (0.90–1.47)		1.14 (1.09–1.20)

[a]Race/ethnic specific multivariate logistic regression analyses were jointly stratified for age group (<30, 5-year age groups to age 79), interviewer and study, and adjusted for menopausal status, age at menarche, hormone therapy use, BMI, income, education, and each of the six factors shown. In analyses on "all subjects," we also jointly stratified by race/ethnicity.

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Table 4. Ovarian cancer PAR%s and 95% CI in Los Angeles County non-Hispanic whites, Hispanics, and African Americans[a]

	Using race-adjusted ORs[a]		
	Non-Hispanic whites PAR%[b]	Hispanics PAR%[b]	African Americans PAR%[b]
No live birth	8.9%	5.7%	5.3%
	5.3%–11.9%	3.4%–7.6%	3.1%–7.0%
No/<1 year oral contraceptives	18.3%	22.2%	15.9%
	12.0%–23.7%	14.5%–28.8%	10.4%–20.7%
No tubal ligation	31.0%	28.7%	27.5%
	17.2%–42.3%	15.9%–39.1%	15.2%–37.5%
Yes endometriosis	4.0%	2.0%	3.4%
	2.0%–5.5%	1.0%–2.8%	1.7%–4.7%
Yes family history ovarian cancer	2.9%	2.7%	3.9%
	1.9%–3.6%	1.8%–3.4%	2.6%–4.9%
Yes/≥1 year talc use	13.0%	12.2%	15.1%
	8.7%–16.8%	8.1%–15.8%	10.0%–19.5%
Three factors (no tubal ligation, no/<1 year oral contraceptives, yes/≥1 year talc use)	50.8%	51.2%	47.9%
	39.7%–59.5%	40.8%–59.3%	37.8%–55.8%
All 6 factors	57.9%	56.1%	53.8%
	48.7%–65.3%	46.8%–63.3%	45.0%–60.7%

[a]Using the all race/ethnicity adjusted ORs from Table 3.
[b]The PARs were mutually adjusted for the variables shown in this table as well as for age group (<30, 5-year age groups to age 79), interviewer and study, menopausal status, age at menarche, hormone therapy use, BMI, income, and education.

it was 90.6% in non-Hispanic whites, 83.8% in Hispanics, and 80.5% in African Americans, so that a shift to the low-risk category, that is, having a tubal ligation, will have a substantial impact. In contrast, the PAR% due to nulliparity is lower because being parous is already highly prevalent; 72.2% in non-Hispanic whites, 83.6% in African Americans, and 82.1% in Hispanics, so that a shift to the low-risk category will have a lesser impact on the overall disease burden.

The mean number of births among parous IEOC cases was 2.5 in non-Hispanic whites, 2.8 in African Americans, and 3.1 in Hispanics (Table 2, bottom). We repeated the PAR% calculations after categorizing births as 0, 1, 2, 3, and 4+ using the 4+ category as baseline: The associated PAR% values increased as expected but the relationships of the PAR%s by racial/ethnic group were essentially unaltered. Similarly, we categorized oral contraceptive use in finer categories of <1 year, 1 to 4 years, 5 to 9 years, and 10+ years with little effect on the relationships of the PAR%s by racial/ethnic group (data not shown).

Discussion

With the high mortality and the lack of effective early screening for ovarian cancer, better understanding of preventive risk factors is a priority. The primary motivation for this analysis was to determine whether the six confirmed nongenetic risk factors for IEOC (parity, use of oral contraceptives, tubal ligation, endometriosis, first-degree family history of ovarian cancer, and use of genital talc) in non-Hispanic whites are also risk factors in Hispanics and African Americans. The risk patterns associated with these six factors were comparable in the three racial/ethnic groups (Table 3), and the PAR%s for the factors jointly (Table 4) were also very similar.

An additional objective was to determine whether these six risk factors jointly could explain the 29% and 15% lower incidence of ovarian cancer in African Americans and Hispanics, respectively, compared with non-Hispanic whites. The incidence of ovarian cancer as reported by SEER, and other cancer registries, is calculated by considering all women in the denominator (population at risk) without removing those who have had a bilateral oophorectomy and are not at risk. Thus, estimates of racial/ethnic differences in IEOC based on SEER data can be "improved" by accounting for the racial/ethnic differences in the prevalence of bilateral oophorectomy.

Although Lowder and colleagues (12) in their analysis of oophorectomy rates in women undergoing a hysterectomy in the National Hospital Discharge Survey covering the period 1979 to 2004, found that the proportion was approximately 40% and did not differ by racial/ethnic group; Jamison and colleagues (13) in their analysis of hysterectomy prevalence in women over age 50 in the Behavioral Risk Factor Surveillance System covering the years 1992 to 2008 found that the rate of hysterectomy was clearly higher in African-American women (47%) than in non-Hispanic whites (41%), and lower still in Hispanic women (36%). Using figures from these two studies in Equation A (see Statistical analysis) to adjust incidence rates for the proportion of women with a history of oophorectomy, we estimate that the observed 29% lower incidence rate in African Americans compared with non-Hispanic whites based on SEER data would be adjusted to 27% $[= 1 - 0.71 \times (1 - 0.41 \times 0.4)/(1 - 0.47 \times 0.4)]$. The PAR% of non-Hispanic whites was slightly higher at 57.8% than the PAR% in African Americans at 53.8% (Table 4); taking this into account, by use of Equation B (see Statistical analysis), reduced the difference in incidence between the two groups further from the adjusted 27% to 20%. Overall, taking into account the correction in the population at risk (denominator) and the PAR%, the difference in the African-American to non-Hispanic white incidence rates was reduced by 31% (1%–20%/29%). Given that hysterectomy rates are lower in Hispanics compared with non-Hispanic whites, Hispanics would be at even lower RR than what is observed in SEER; the 15% lower incidence rate in Hispanics compared with non-Hispanic whites would increase to 17% when using the correct at-risk denominator. The PAR% difference will change the difference slightly less in Hispanics compared with non-Hispanic whites from 17% to 13%. When taking into consideration the correct population at risk and the PAR%, the difference in incidence rates between Hispanics and non-Hispanic

whites is reduced by 13% (1%–13%/15%). Thus, this type of analysis suggests that further investigations are needed to identify other risk factors that may explain the remaining differences in IEOC rates between these three racial/ethnic groups.

Strengths of this study include the ability to evaluate the relative comparability in the effect of several well-established risk factors in non-Hispanics whites, Hispanics, and African Americans. Our results on Hispanics fill a knowledge gap, as this is the first study to examine etiologic risk factors for ovarian cancer in this growing minority population in the United States. Identical questionnaires and protocols were used in these four studies. Although information on these six factors was based on self-report, there is no evidence of systematic misclassification bias as the direction of racial/ethnic differences in the prevalence of tubal ligation, use of oral contraceptives, and endometriosis are consistent with other studies (6, 14–16). However, these results must be considered with caution as we were limited in that the sample sizes of Hispanics and African Americans were modest, and we investigated only the six factors that are confirmed, noncontroversial, showing strong associations with all invasive ovarian cancers in non-Hispanic whites. The modest sample sizes precluded us from conducting analyses separately by histologic type. The response rate for the three racial/ethnic groups was also modest, but not unlike the response rate for other case–control studies on ovarian cancer.

The comparable risk factor associations in IEOC in African Americans, Hispanics, and non-Hispanic whites contrast sharply with the more disparate risk factor patterns in breast cancer by race/ethnicity. A number of factors that are known to affect breast cancer risk in non-Hispanic whites do not appear to influence risk in African Americans and these differences cannot be explained by different prevalence of estrogen receptor/progesterone receptor–positive breast tumors between the two groups (17–21). Breast cancer risk factors also appeared to differ profoundly between Hispanics and non-Hispanic whites in one of the few studies with comparable data on both race/ethnic groups (15). Given the more comparable risk factor patterns in IEOC for non-Hispanic whites, Hispanics, and African Americans, devising strategies to lessen the burden of IEOC will be applicable to all groups.

Summary

Results from these population-based case–control studies suggest that the six well-established risk factors for IEOC accounted for about 60% of ovarian cancer risk in non-Hispanic whites, Hispanics, and African Americans. There were differences in the prevalence of these factors in the different racial/ethnic groups, and the 27% lower incidence of ovarian cancer in African Americans compared with non-Hispanic whites was reduced to 20% when these differences were adjusted for, but adjustment for these differences in prevalence accounted for only a very small amount of the lower incidence rate in Hispanics.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The ideas and opinions expressed herein are those of the authors, and endorsement by the State of California, the California Department of Health Services, the National Cancer Institute, or the Centers for Disease Control and Prevention or their contractors and subcontractors is not intended nor should be inferred.

Authors' Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.H. Wu, M.C. Pike
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.H. Wu, C.L. Pearce, C.-C. Tseng, M.C. Pike
Writing, review, and/or revision of the manuscript: A.H. Wu, C.L. Pearce, M.C. Pike
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.H. Wu
Study supervision: A.H. Wu, M.C. Pike

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Cancer Epidemiology, Biomarkers & Prevention

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African Americans and Hispanics Remain at Lower Risk of Ovarian Cancer Than Non-Hispanic Whites after Considering Nongenetic Risk Factors and Oophorectomy Rates

Anna H. Wu, Celeste L. Pearce, Chiu-Chen Tseng, et al.

Cancer Epidemiol Biomarkers Prev 2015;24:1094-1100. Published OnlineFirst April 14, 2015.

Exhibit 49

OPEN

The Association Between Talc Use and Ovarian Cancer

A Retrospective Case–Control Study in Two US States

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Background: Multiple studies of ovarian cancer and genital talc use have led only to consensus about possible carcinogenicity. Seeking greater clarity, we examined this association in 2,041 cases with epithelial ovarian cancer and 2,100 age- and-residence-matched controls.
Methods: We defined genital talc use as regular application to the genital/rectal area directly, on sanitary napkins, tampons, or underwear. To estimate "talc-years," we multiplied applications per year by years used. Unconditional logistic regression, Wald statistics, likelihood-ratio tests, and polytomous logistic regression were used to calculate adjusted odds ratios (OR) and 95% confidence intervals (CI), trends, effect-modification, and heterogeneity by ovarian cancer histologic subtype.
Results: Overall, genital talc use was associated with an OR (95% CI) of 1.33 (1.16, 1.52), with a trend for increasing risk by talc-years. Women who used talc were more likely to be older, heavier, asthma sufferers, and regular analgesic users—none of which was a confounder. Dose–responses were more apparent for premenopausal women, especially nonsmokers and those heavier or postmenopausal users of menopausal hormones (hormone therapy [HT]). Subtypes of ovarian cancer more likely to be associated with talc included invasive serous and endometrioid tumors and borderline serous and mucinous tumors. Premenopausal women and postmenopausal HT users with these subtypes who had accumulated >24 talc-years had ORs (95% CI) of 2.33 (1.32, 4.12) and 2.57 (1.51, 4.36), respectively.
Conclusion: Risks for epithelial ovarian cancer from genital talc use vary by histologic subtype, menopausal status at diagnosis, HT use, weight, and smoking. These observations suggest that estrogen and/or prolactin may play a role via macrophage activity and inflammatory response to talc.

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In the 1960s, a link between talc and ovarian cancer was suggested by observations that some talc powders contained asbestos[1] and that asbestos placed intraperitoneally in animals transformed the single layer of the ovarian surface to a multilayered one with abnormal cells.[2] A 1971 study found particles compatible with talc in human ovarian and uterine cancers.[3] A 1982 case–control study was the first to link genital talc use with ovarian cancer.[4] Dozens more followed confirming the association including some larger ones cited here.[5–13] The most recent meta-analysis reported a summary odds ratio (OR) and 95% confidence interval (CI) of 1.35 (1.26, 1.46).[14] In 2006, the International Agency for Research on Cancer declared that talc used genitally is possibly carcinogenic.[15] However, a study with null results from the Women's Health Initiative (WHI)[16] and accompanying editorial[17] cast new skepticism on the association. Here, we present data from combined phases of a case–control study of ovarian cancer involving more than 4,000 women to provide fresh perspectives on this association.

METHODS

Study Population

Data come from three enrollment phases: 1 (1992–1997), 2 (1998–2002), and 3 (2003–2008). Articles we previously published related to talc include a detailed report from phase 1,[7] data from phases 1 and 2 combined with Nurses' Health Study data,[18] and phases 1–3 data combined with data from several participants in the Ovarian Cancer Association Consortium (OCAC).[19] This is the first detailed examination of talc data from the combined phases of our study.

Details regarding enrollment are described elsewhere.[20] In brief, 3,957 women residing in Eastern Massachusetts and New Hampshire diagnosed with ovarian cancer between ages 18 and 80 were identified through tumor boards and registries.

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Eight hundred and seventy-four cases were ineligible if they had died, moved outside study area, did not have a working telephone number, or had a nonovarian primary tumor. Of the remaining 3,083 cases, 2,203 (71%) were enrolled. Excluding 127 nonepithelial and 35 mixed mesodermal tumors, 2,041 cases with epithelial tumors of ovarian, primary peritoneal, and Fallopian tube origin, including borderline malignancies (henceforth, epithelial ovarian cancer) were included. Pathology reports were reviewed and histologic subtype, grade, and stage recorded. Mixed epithelial ovarian cancer was classified as the predominant type. Undifferentiated, transitional cell, fallopian tube, or primary peritoneal tumors were counted as serous.[21] Other mixed epithelial (n = 102), malignant Brenner (n = 5), and unspecified epithelial tumors (n = 27) were classified as other.

Controls were identified through random digit dialing, driver-license lists, and town-resident lists. Between 1992 and 1997, 420 (72%) identified through random digit dialing and 102 (51%) through lists agreed to participate. From 1998 to 2008, 4,366 potential controls were identified using the lists, of whom 1,426 (33%) were ineligible if they had died, moved, or were seriously ill or if they did not have a working telephone, speak English, or have ovaries. Of eligible controls, 1,362 (46%) declined to participate by phone or via "opt-out" postcard and 1,578 (54%) were enrolled (2,100 total). Controls were frequency matched to cases by 5-year age groups and region of residence.

Exposure Assessment

Subjects were personally interviewed about potential ovarian cancer risk factors that occurred more than 1 year before diagnosis, for cases, and interview, for controls. Subjects were asked whether they "regularly" or "at least monthly" applied powder to the genital or rectal area, sanitary napkins or tampons, underwear, or areas other than the genital-rectal area. Additional details included type of powder, age begun, years used, and applications per month. Lifetime exposure was estimated by multiplying frequency of applications per month by months used. This was divided by 360 (i.e., daily use coded as 30/month) to yield talc-years. To create categorical variables for talc-years, we chose cut points based on quartiles for exposed controls and rounded to the nearest integer. In addition, we asked participants if their partners dusted or sprayed powder to their genital or rectal areas. Condom and diaphragm use as potential sources of talc exposure were also recorded.

We calculated ovulatory cycles by subtracting age at menarche from age at last period, reduced this by time spent pregnant, breastfeeding, or using oral contraceptives, and dividing the remainder by each woman's average cycle length. Family history was defined as a mother or sister with ovarian or premenopausal breast cancer. Women who reported postmenopausal hormone use were classified as hormone therapy (HT) users and type(s) of HT was recorded. Participants completed a food-frequency questionnaire[22] from which grams of alcohol consumed per day were estimated.

Statistical Methods

Unconditional logistic regression was used to model the OR and 95% CI adjusted first for matching factors (age, study center, and phase) and then fully by potential confounders. Likelihood ratio tests comparing models with and without interaction terms were used to test for effect modification. Tests for trend were based on the Wald statistic using continuous variables weighted by category midpoints with zero assigned as the exposure for nonusers. Polytomous logistic regression was used to simultaneously estimate separate ORs and 95% CIs for genital talc use by histologic subtypes. Likelihood-ratio tests were used to calculate *P* values for heterogeneity by comparing polytomous logistic regression models in which the talc association was held constant over case subgroups to models that allowed the association to differ between case subgroups.[23] Analyses were performed using SAS v9.3 (SAS Institute, Cary, NC) and polytomous logistic regression analyses were performed in Stata (StataCorp LP, College Station, TX). Sensitivity analyses to assess the influence of exposure misclassification were performed with Excel using quantitative analysis methods described previously.[24]

Ethical Approval

Institutional review boards approved the study. All participants provided written informed consent.

RESULTS

Genital use of talc, either alone or in combination with body use, was associated with elevated epithelial ovarian cancer risk (Table 1). Among women with no personal use, there was no increased risk with potential exposure from diaphragms, condoms, or partner use. Therefore, only those with personal genital talc exposure were classified as ever-users. Genital talc use was associated with an OR (95% CI) of 1.33 (1.16, 1.52) adjusted only for age, study center, and phase. Most women reported using Johnson & Johnson's Baby Powder or Shower to Shower. Fourteen women who reported exclusive use of a cornstarch-based powder were considered unexposed. The average age women began using talc was 20.0 for cases and 19.8 for controls. Almost half of users were currently using or had only recently discontinued powder use at the reference date. Risk decreased with increased time since last use. The trend for frequency of use was significant, but the trend for years used was flat. Some subjects reported they used talc only seasonally, but our original questionnaire did not capture this detail. A question to capture months-per-year-used was added in 1998 and was available for 54% of cases and 56% of controls. Year-round use was the most common pattern, and more cases than controls used powder year-round. ORs for talc-years among those who reported months-per-year-used are shown as the next-to-final entry in Table 1. An OR of 1.49 (95% CI = 1.06, 2.10) was associated with more

TABLE 1. Type, Timing, and Duration of Genital Talc Use

	Control Subjects N (%)	Case Subjects N (%)	Adjusted[a] OR (95% CI)
Personal use			
None	1,099 (52)	1,001 (49)	1.00 (referent)
Body use only	452 (22)	398 (20)	0.99 (0.84, 1.16)
Genital use only	74 (4)	94 (5)	1.42 (1.04, 1.96)
Body and genital use	475 (23)	548 (27)	1.30 (1.12, 1.52)
Potential exposure in women with no personal use			
None	447 (41)	461 (46)	1.00 (referent)
Diaphragm only	207 (19)	155 (15)	0.73 (0.57, 0.93)
Condoms, with or without diaphragm	367 (33)	308 (31)	0.82 (0.66, 1.01)
Partner use, with or without diaphragm or condoms	78 (7)	77 (8)	0.96 (0.68, 1.35)
Any genital powder use			
No	1,551 (74)	1,399 (69)	1.00 (referent)
Yes	549 (26)	642 (31)	1.33 (1.16, 1.52)
Type of genital powder used			
No genital use	1,542 (73)	1,394 (68)	1.00 (referent)
Cornstarch use only	9 (<1)	5 (<1)	0.58 (0.19, 1.74)
Johnson and Johnson Baby Powder or Shower to Shower	316 (15)	363 (18)	1.30 (1.10, 1.54)
Other brand(s)	233 (11)	279 (14)	1.35 (1.12, 1.64)
Age first used genital powder[b]			
Never used	1,551 (74)	1,399 (69)	1.00 (referent)
<20	343 (16)	363 (18)	1.19 (1.01, 1.41)
20–29	122 (6)	183 (9)	1.71 (1.34, 2.17)
≥30	76 (4)	87 (4)	1.31 (0.95, 1.80)
Time since exposure ended			
No genital use	1,551 (74)	1,399 (69)	1.00 (referent)
≥35 years	51 (2)	52 (3)	1.18 (0.79, 1.75)
25–34 years	81 (4)	88 (4)	1.24 (0.91, 1.70)
15–24 years	72 (3)	82 (4)	1.30 (0.94, 1.80)
5–14 years	79 (4)	95 (5)	1.36 (1.00, 1.85)
Currently using or recently stopped	255 (12)	314 (15)	1.38 (1.15, 1.65)
P trend			<0.0001
Frequency of use			
No genital use	1,551 (74)	1,399 (69)	1.00 (referent)
1–7 days per month	220 (11)	227 (11)	1.17 (0.96, 1.44)
8–29 days per month	110 (5)	133 (7)	1.37 (1.05, 1.78)
≥30 days per month	205 (10)	267 (13)	1.46 (1.20, 1.78)
P trend			<0.0001
Years used			
Never used	1,551 (74)	1,399 (69)	1.00 (referent)
<8	133 (6)	152 (8)	1.31 (1.03, 1.68)
8–19	126 (6)	145 (7)	1.31 (1.02, 1.68)
20–35	147 (7)	178 (9)	1.35 (1.07, 1.70)
>35	129 (6)	152 (7)	1.33 (1.03, 1.71)
P trend			0.002
Months per year of use[c]			
No genital use	1,551 (83)	1,399 (80)	1.00 (referent)
1–3 months per year	61 (3)	60 (3)	1.11 (0.77, 1.61)
4–11 months per year	55 (3)	56 (3)	1.13 (0.77, 1.66)
12 months per year	193 (10)	229 (13)	1.35 (1.09, 1.67)
P trend			0.006

(*Continued*)

TABLE 1. (*Continued*)

	Control Subjects N (%)	Case Subjects N (%)	Adjusted[a] OR (95% CI)
Total genital talc applications (apps) among only those who reported months per year of use[c]			
No genital use	1,551 (83)	1,399 (80)	1.00 (referent)
≤360 apps (equivalent to 1 year of daily use)	106 (6)	103 (6)	1.10 (0.83, 1.47)
361–1,800 apps (equivalent to >1–5 years of daily use)	79 (4)	96 (5)	1.38 (1.01, 1.88)
1,801–7,200 apps (equivalent to >5–20 years of daily use)	61 (3)	63 (4)	1.16 (0.80, 1.66)
>7,200 apps (equivalent to >20 years of daily use)	63 (3)	83 (5)	1.49 (1.06, 2.10)
P trend			0.02
Total genital talc applications among all (assuming 12 months/year when missing months per year of use)			
No genital use	1,551 (74)	1,399 (69)	1.00 (referent)
≤360 apps (equivalent to 1 year of daily use)	138 (7)	138 (7)	1.15 (0.89, 1.47)
361–1,800 apps (equivalent to >1–5 years of daily use)	124 (6)	148 (7)	1.36 (1.06, 1.75)
1,801–7,200 apps (equivalent to >5–20 years of daily use)	124 (6)	156 (8)	1.41 (1.10, 1.80)
>7,200 apps (equivalent to >20 years of daily use)	149 (7)	185 (9)	1.39 (1.11, 1.75)
P trend			0.003

[a]Adjusted only for the study matching factors: reference age, study center, and study phase.
[b]Nine cases and nine controls reported they knew that talc had been used on them in infancy so their age at exposure was recorded as 1.
[c]Excludes talc users from phase 1 and part of phase 2 because months/year of use was not collected.

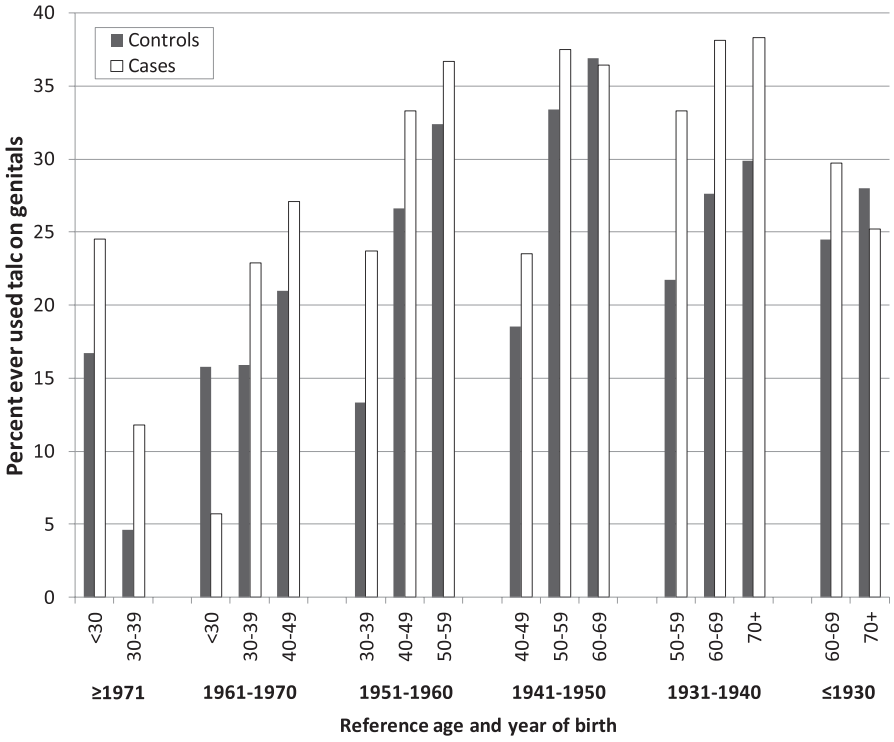

FIGURE 1. Proportions of cases and controls who ever used talc on genitals in categories by decade of birth and reference age.

than 20 talc-years (>7,200 applications) and a dose–response. For subjects missing the seasonal-use variable, we assumed 12 months per year in calculating talc-years in the final entry in Table 1, as well as in subsequent tables and figures examining talc-years. Even with this imprecision, the trend remained, although the increase was less monotonic.

Figure 1 shows the proportions of cases and controls who used talc in the genital area by decade of birth and age at diagnosis or interview. In 13 of the 16 age-and-birth categories, a greater proportion of cases used talc compared with controls. This suggests that the association between genital use of talc and epithelial ovarian cancer is not confined to any particular age or birth cohort.

Powder users, both cases and controls, were more likely to be older, heavier, asthma sufferers, and regular analgesic users (Table 2). By tests for interaction (column 3), the

TABLE 2. Illustrating Potential Effect Modification and Confounding

	Controls		Cases				
	No Genital Talc Use N (%)	Any Genital Talc Use N (%)	No Genital Talc Use N (%)	Any Genital Talc Use N (%)	Stratum-specific OR (95% CI)[a] for Genital Talc Use	*P* Int[b]	OR (95% CI) for Genital Talc Use Adjusted[c]
Age							
<50	670 (80)	165 (20)	600 (74)	211 (26)	1.42 (1.13, 1.80)	0.63	1.30 (1.13, 1.49)[d]
50–64	599 (68)	278 (32)	541 (64)	308 (36)	1.25 (1.03, 1.53)		
≥65	282 (73)	106 (27)	258 (68)	123 (32)	1.35 (0.98, 1.85)		
Study center							
New Hampshire	319 (82)	72 (18)	316 (74)	109 (26)	1.52 (1.08, 2.14)	0.30	1.31 (1.15, 1.50)[e]
Massachusetts	1,232 (72)	477 (28)	1,083 (67)	533 (33)	1.29 (1.11, 1.50)		
Study phase							
1	430 (82)	92 (18)	409 (73)	149 (27)	1.71 (1.27, 2.30)	0.12	1.33 (1.16, 1.52)[f]
2	519 (72)	202 (28)	448 (68)	210 (32)	1.23 (0.97, 1.55)		
3	602 (70)	255 (30)	542 (66)	283 (34)	1.25 (1.02, 1.54)		
Race							
White	1,500 (74)	531 (26)	1,321 (68)	612 (32)	1.35 (1.17, 1.55)	0.002	1.33 (1.16, 1.53)
African American	17 (74)	6 (26)	16 (46)	19 (54)	5.08 (1.32, 19.6)		
Hispanic	27 (82)	6 (18)	25 (81)	6 (19)	1.10 (0.30, 4.12)		
Asian	5 (50)	5 (50)	34 (94)	2 (6)	0.04 (0.01, 0.34)		
Other	2 (67%)	1 (33)	3 (50)	3 (50)	-		
Body mass index							
<24.9	798 (76)	251 (24)	727 (72)	284 (28)	1.25 (1.03, 1.53)	0.59	1.32 (1.15, 1.51)
≥24.9	753 (72)	298 (28)	672 (65)	358 (35)	1.38 (1.14, 1.67)		
Height (m)							
<1.63	755 (73)	283 (27)	689 (68)	325 (32)	1.28 (1.06, 1.56)	0.71	1.32 (1.16, 1.52)
≥1.63	795 (75)	266 (25.)	710 (69)	317 (31)	1.37 (1.13, 1.66)		
Weight (lbs)							
<148	799 (77)	241 (23)	727 (73)	272 (27)	1.24 (1.01, 1.52)	0.58	1.32 (1.15, 1.51)
≥148	745 (71)	307 (29)	670 (64)	370 (36)	1.38 (1.15, 1.66)		
Parity							
Nulliparous	284 (75)	94 (25)	455 (70)	195 (30)	1.28 (0.96, 1.71)	0.71	1.33 (1.15, 1.52)
Parous	1,267 (74)	455 (26)	944 (68)	447 (32)	1.34 (1.15, 1.57)		
Ever breastfed							
No	781 (72)	296 (28)	953 (69)	430 (31)	1.21 (1.01, 1.45)	0.16	1.30 (1.13, 1.50)
Yes	770 (75)	253 (25)	446 (68)	212 (32)	1.48 (1.19, 1.85)		
Oral contraceptive use							
Never or <3 months	559 (73)	207 (27)	672 (69)	302 (31)	1.25 (1.01, 1.55)	0.38	1.32 (1.15, 1.51)
≥3 months	992 (74)	342 (26)	727 (68)	340 (32)	1.39 (1.16, 1.67)		
Intrauterine device use							
No	1,300 (74)	447 (26)	1,203 (69)	547 (31)	1.35 (1.16, 1.56)	0.59	1.33 (1.16, 1.52)
Yes	251 (71)	102 (29)	196 (67)	95 (33)	1.20 (0.85, 1.70)		
Ovulatory cycles							
<366	748 (78)	214 (22)	542 (74)	191 (26)	1.28 (1.02, 1.61)	0.76	1.31 (1.14, 1.52)
≥366	680 (71)	281 (29)	733 (65)	402 (35)	1.37 (1.13, 1.65)		
Endometriosis or painful periods							
No	1,006 (74)	345 (26)	814 (70)	351 (30)	1.29 (1.08, 1.55)	0.77	1.31 (1.14, 1.50)
Yes	545 (73)	204 (27)	585 (67)	291 (33)	1.35 (1.09, 1.67)		
Jewish ethnicity							
No	1,455 (74)	518 (26)	1,277 (69)	585 (31)	1.33 (1.15, 1.53)	0.72	1.33 (1.16, 1.52)
Yes	96 (76)	31 (24)	122 (68)	57 (32)	1.39 (0.83, 2.33)		

(*Continued*)

TABLE 2. (*Continued*)

	Controls		Cases				
	No Genital Talc Use N (%)	Any Genital Talc Use N (%)	No Genital Talc Use N (%)	Any Genital Talc Use N (%)	Stratum-specific OR (95% CI)[a] for Genital Talc Use	*P* Int[b]	OR (95% CI) for Genital Talc Use Adjusted[c]
Family history[g]							
No	1,446 (74)	510 (26)	1,267 (68)	585 (32)	1.34 (1.16, 1.55)	0.61	1.33 (1.16, 1.52)
Yes	105 (73)	39 (27)	132 (70)	57 (30)	1.19 (0.73, 1.93)		
Personal history of breast cancer							
No	1,498 (74)	519 (26)	1,299 (68)	606 (32)	1.38 (1.20, 1.59)	0.01	1.33 (1.16, 1.53)
Yes	53 (64)	30 (36)	100 (74)	36 (26)	0.67 (0.37, 1.22)		
Hysterectomy or tubal ligation							
No	1,135 (74)	401 (26)	1,134 (70)	480 (30)	1.22 (1.04, 1.43)	0.02	1.34 (1.16, 1.53)
Yes	416 (74)	148 (26)	265 (62)	162 (38)	1.73 (1.31, 2.27)		
Menopausal status and HT							
Premenopausal	735 (79)	197 (21)	653 (73)	247 (27)	1.41 (1.13, 1.75)	<0.001	1.33 (1.16, 1.53)
Postmenopausal, no HT	507 (69)	230 (31)	549 (70)	238 (30)	0.97 (0.78, 1.20)		
Postmenopausal, HT	309 (72)	122 (28)	197 (56)	157 (44)	2.21 (1.63, 3.00)		
Current smoking							
No	1,332 (74)	473 (26)	1,149 (68)	538 (32)	1.35 (1.16, 1.56)	0.60	1.32 (1.16, 1.52)
Yes	219 (74)	76 (26)	250 (71)	104 (29)	1.19 (0.84, 1.69)		
Ever smoked							
No	759 (75)	248 (25)	669 (70)	291 (30)	1.34 (1.10, 1.64)	0.72	1.32 (1.16, 1.52)
Yes	792 (72)	301 (28)	730 (68)	351 (32)	1.31 (1.09, 1.58)		
Asthma							
No	1,442 (75)	492 (25)	1,310 (69)	586 (31)	1.34 (1.16, 1.55)	0.70	1.33 (1.16, 1.52)
Yes	109 (66)	57 (34)	89 (61)	56 (39)	1.25 (0.78, 2.01)		
Alcohol (grams per day)							
≤2.32	753 (74)	269 (26)	738 (70)	311 (30)	1.19 (0.98, 1.45)	0.29	1.30 (1.13, 1.50)
>2.32	763 (75)	259 (25)	623 (68)	291 (32)	1.43 (1.17, 1.75)		
Any acetaminophen use							
No	1,190 (76)	373 (24)	1,076 (71)	431 (29)	1.30 (1.10, 1.53)	0.83	1.32 (1.15, 1.52)
Yes	361 (67)	176 (33)	323 (60)	211 (40)	1.41 (1.09, 1.82)		
Any aspirin or ibuprofen use							
No	936 (77)	285 (23)	901 (71)	361 (29)	1.32 (1.10, 1.59)	0.94	1.34 (1.17, 1.53)
Yes	615 (70)	264 (30)	498 (64)	281 (36)	1.36 (1.11, 1.68)		
Adjusted for all variables	1,551	549	1,399	642	-	-	1.32 (1.15, 1.53)

[a]Adjusted for reference age (continuous), study center, and study phase.
[b]*P* for interaction from likelihood ratio tests comparing models with main effects and interaction terms to models with main effects only.
[c]Adjusted for reference age (continuous), study center, study phase, and each variable listed (individually). BMI, height, weight, and ovulatory cycles were adjusted for with indicators for quartiles and parity (nulliparous, 1, 2, ≥2), breastfeeding (never, <4, 4–9, 10–19, >19 months), and OC use (never, <23, 23–49, 50–96, >96 months) were adjusted for with indicators for categories.
[d]Adjusted for reference age only.
[e]Adjusted for reference age and study center.
[f]Adjusted for reference age, study center, and study phase.
[g]Family history of ovarian or early onset breast cancer in a mother or sister.

association was significantly greater for women who were African American, had no personal history of breast cancer, had a tubal ligation or hysterectomy, were premenopausal, or were postmenopausal and used HT. The latter finding, together with the dose–response data, is illustrated in Figure 2. Among the HT users, 92% used estrogen (alone or in combination), 2% used progesterone alone, and 5% used creams or suppositories. Increased epithelial ovarian cancer risk with genital talc use was found in both women who had used estrogen alone or estrogen plus progesterone. Too few women used progesterone only HT or estrogen creams or suppositories to examine risk with talc use in these groups (data not shown). The median duration of HT use was 5 years. Subjects with <5 years of HT use had an overall OR (95% CI) for EOC risk with ever-use of talc on genitals of 2.93 (1.86, 4.62). Subjects with ≥5 years of HT use had an OR (95% CI) that was slightly lower, 1.73

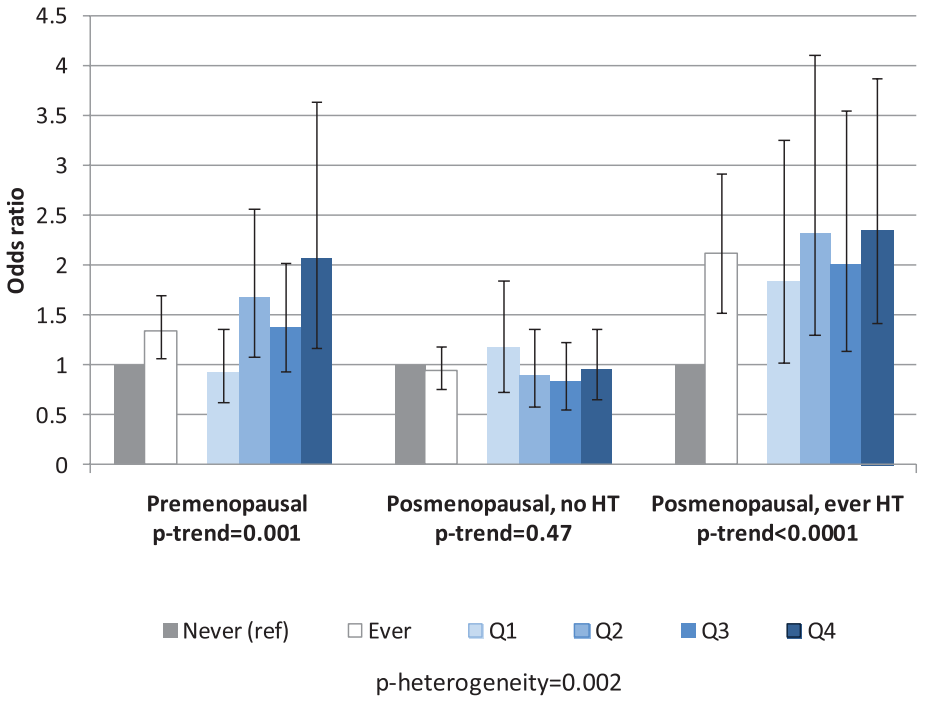

FIGURE 2. Associations between use of talc on genitals (never/ever and quartiles of talc-years) and ovarian cancer by menopausal status and postmenopausal hormone therapy.

(1.15, 2.62), but a clearer trend for increasing risk with talc-years was more apparent in the longer term HT users (data not shown). To explore the potential interaction between talc use and hysterectomy or tubal ligation, we restricted this analysis to subjects who had either or both procedures (Table 3). For premenopausal women, risk for EOC was increased in women who used talc before the procedure, while risk was elevated for use both before and after the procedure in postmenopausal women who used HT. No associations were seen in postmenopausal women who had not used HT. There were too few subjects who had used talc only after a hysterectomy or tubal ligation to permit reliable estimates of risk.

Returning to Table 2, we applied the convention that a variable may be a confounder if adjustment yields a 10% difference compared with the crude OR (or, in our study, compared with the OR of 1.33 adjusted for age, study center, and study phase). A 10% lower or greater change corresponds to an OR ≤1.20 or ≥1.46. As seen in the far right column, the OR of 1.33 for ovarian cancer risk was not materially changed after adjustment for any individual or all variables.

Because Figure 2 suggests that EOC risk with talc varies by menopausal status, we revisited the issue of interaction in eTable 1 (http://links.lww.com/EDE/B2) in which subjects are stratified by menopausal status. Although few significant interactions were seen, categories for several variables revealed contrasting overall associations and/or clearer dose–responses (Fig. 3). For premenopausal women, these included women with a BMI > 25, those who had breastfed, those who were not current smokers, and those who consumed more than 2.32 g of alcohol per day. In addition, the association was stronger for both pre- and post-menopausal women who were least likely to have a genetic basis for their ovarian cancer, defined as women with no personal history of breast cancer, without a primary relative with either ovarian cancer or premenopausal breast cancer, and non-Jewish women (eTable 1; http://links.lww.com/EDE/B2). No important interactions were observed for postmenopausal women, except for weight and BMI, HT use, and the combined "genetic" variable.

Table 4 shows ORs stratified by menopausal status and histologic subtype of epithelial ovarian cancer. Overall, talc use increased risk for serous and endometrioid invasive and serous borderline tumors with the dose–response most apparent for serous invasive cancer. For premenopausal women, both the overall associations and dose–responses were stronger with serous invasive and serous borderline tumors. Premenopausal women also had an increased risk for mucinous borderline tumors at the highest quartile of talc use OR = 2.28 (1.23, 4.26) and a dose–response. For postmenopausal women, dose–responses were strongest for women with invasive serous and endometrioid tumors. Talc use was not associated with clear cell or mucinous invasive epithelial ovarian cancer regardless of menopausal status. The ORs and dose–responses for the combined histologic subtypes relevant to pre- and post-menopausal women are shown in Table 5. Except for a few categories, these were not materially different than those illustrated in Figure 2. However, notably, premenopausal women and postmenopausal HT-users with the relevant subtypes who had accumulated >24 talc-years had ORs (95% CI) of 2.33 (1.32, 4.12) and 2.57 (1.51, 4.36), respectively.

TABLE 3. Effect of Tubal Ligation and Hysterectomy by Menopausal Status and Hormone Therapy on Association Between Genital Talc Use and Ovarian Cancer

Genital Talc Use Among Women Who Had a Hysterectomy or Tubal Ligation[a]	Premenopausal			Postmenopausal, Never Used HT			Postmenopausal, Ever Used HT		
	Controls	Cases	Adjusted[b]	Controls	Cases	Adjusted[b]	Controls	Cases	Adjusted[b]
	N (%)	N (%)	OR (95% CI)	N (%)	N (%)	OR (95% CI)	N (%)	N (%)	OR (95% CI)
Never used	147 (79)	94 (71)	1.00 (referent)	139 (67)	113 (67)	1.00 (referent)	130 (77)	58 (48)	1.00 (referent)
Used both before and after	26 (14)	17 (13)	0.99 (0.48, 2.06)	45 (22)	36 (21)	1.00 (0.58, 1.72)	21 (13)	40 (33)	5.85 (2.88, 11.9)
Used before only	10 (5)	20 (15)	4.40 (1.73, 11.2)	20 (10)	16 (10)	0.99 (0.46, 2.10)	12 (7)	18 (15)	3.49 (1.39, 8.75)
Used after only	3 (2)	1 (1)	0.33 (0.03, 3.60)	3 (1)	4 (2)	1.66 (0.34, 8.21)	5 (3)	5 (4)	2.11 (0.49, 9.17)

[a]The median ages for tubal ligation and hysterectomy, respectively, were 34 and 39 for cases and 34 and 40 for controls.
[b]Adjusted for reference age (continuous), study center, study phase (3, 4, 5), parity (nulliparous, 1, 2, ≥2), breastfeeding (never, <4, 4–9, 10–19, >19 months), OC use (never, <23, 23–49, 50–96, >96 months), IUD (never, ever), endometriosis or painful periods, personal history of breast cancer, Jewish ethnicity, tubal ligation, and BMI (<22.2, 22.2–24.8, 24.9–28.6, >28.6).

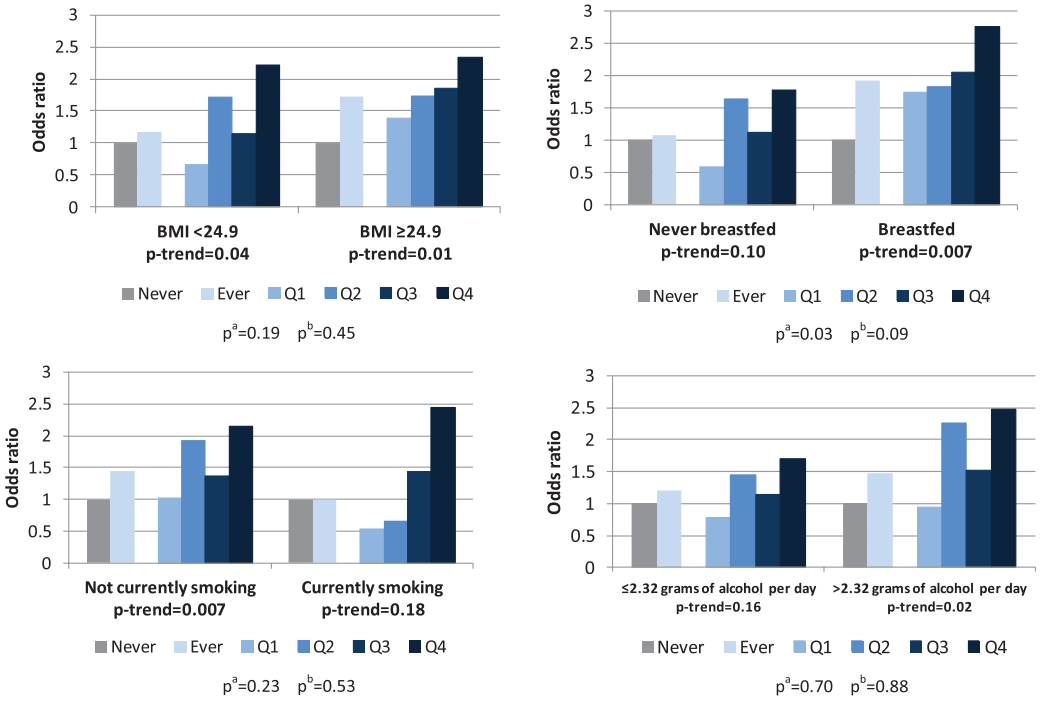

FIGURE 3. Variables modifying the talc association in premenopausal women. [a]*P* heterogeneity from likelihood ratio tests comparing a model with ever/never talc use and the effect modifier to a model with these plus the interaction term between them. [b]*P* heterogeneity from likelihood ratio tests comparing a model with indicators for each quartile of talc-years and the effect modifier to a model with these plus their interacton terms.

DISCUSSION

We analyzed case–control data collected over 16 years on talc use and epithelial ovarian cancer risk to address issues related to definition of the exposure, bias and confounding, effect modification, histologic heterogeneity, and dose–response. Talc used regularly in the genital area was associated with a 33% increase in ovarian cancer risk overall while no apparent risk was associated with talc used only in nongenital areas. Our results are consistent with a recent pooled analysis from the OCAC which reported that use of powder on genitals is associated with a 24% increased risk and no effect of nongenital use of talc.[19] There was general agreement on risk by histologic type of epithelial ovarian cancer except that OCAC found an association with clear cell cancer and we did not. The findings from OCAC and our study contrast with null results from the WHI cohort analysis[17] raising the issue of recall bias in case–control studies.

Addressing recall bias, we conducted a sensitivity analysis that assumed truly nonexposed cases and controls were accurately classified as unexposed (i.e., specificity 99%) and

TABLE 4. Genital Talc Applications by Histologic Type and Menopausal Status

		Serous Invasive		Mucinous Invasive		Endometrioid Invasive		Clear Cell Invasive			Serous Borderline		Mucinous Borderline		
Characteristic	Controls %	Cases %	Adjusted[a] OR (95% CI)	Cases %	Adjusted[a] OR (95% CI)	Cases %	Adjusted[a] OR (95% CI)	Cases %	Adjusted[a] OR (95% CI)	*P* Het	Cases %	Adjusted[a] OR (95% CI)	Cases %	Adjusted[a] OR (95% CI)	*P* Het
All women	N = 2,100	N = 968		N = 95		N = 327		N = 114			N = 250		N = 147		
No use	74	65	1.00	78	1.00	67	1.00	74	1.00	0.13	70	1.00	78	1.00	0.20
Any use	26	35	1.42 (1.19, 1.69)	22	0.87 (0.53, 1.44)	33	1.38 (1.06, 1.80)	26	1.01 (0.65, 1.57)		30	1.40 (1.03, 1.90)	22	1.02 (0.67, 1.54)	
No genital use	74	66	1.00	78	1.00	67	1.00	74	1.00		70	1.00	78	1.00	
≤1 talc-year	7	7	1.30 (0.94, 1.79)	8	1.30 (0.60, 2.82)	8	1.30 (0.81, 2.07)	6	0.94 (0.42, 2.14)		8	1.38 (0.80, 2.36)	2	0.33 (0.10, 1.06)	
>1–5 talc-years	6	7	1.45 (1.05, 2.01)	3	0.57 (0.18, 1.85)	8	1.54 (0.96, 2.48)	7	1.44 (0.66, 3.13)		8	1.72 (1.02, 2.89)	6	1.31 (0.63, 2.71)	
>5–24 talc-years	7	9	1.33 (0.99, 1.79)	8	1.15 (0.54, 2.46)	8	1.14 (0.72, 1.80)	5	0.63 (0.27, 1.51)		8	1.18 (0.69, 2.00)	11	1.64 (0.92, 2.92)	
>24 talc-years	6	11	1.54 (1.15, 2.07)	2	0.38 (0.09, 1.60)	9	1.67 (1.06, 2.63)	8	1.35 (0.64, 2.84)		6	1.55 (0.87, 2.77)	3	0.84 (0.32, 2.16)	
P trend			0.003		0.24		0.04		0.64	0.16		0.16		0.76	0.55
Premenopausal	N = 932	N = 282		N = 51		N = 177		N = 56			N = 175		N = 108		
No use	79	70	1.00	78	1.00	70	1.00	79	1.00	0.49	72	1.00	78	1.00	0.44
Any use	21	30	1.43 (1.04, 1.98)	22	1.04 (0.52, 2.10)	30	1.34 (0.91, 1.98)	21	0.87 (0.44, 1.75)		28	1.56 (1.06, 2.31)	22	1.25 (0.75, 2.06)	
No genital use	79	70	1.00	78	1.00	71	1.00	79	1.00		72	1.00	78	1.00	
≤1 talc-year	7	5	0.71 (0.38, 1.34)	14	1.81 (0.75, 4.37)	9	1.13 (0.60, 2.11)	4	0.33 (0.08, 1.47)		9	1.51 (0.82, 2.80)	1	0.13 (0.02, 0.99)	
>1–5 talc-years	5	7	1.71 (0.94, 3.12)	4	1.01 (0.23, 4.42)	7	1.58 (0.77, 3.27)	9	2.41 (0.83, 7.01)		7	1.58 (0.78, 3.21)	6	1.57 (0.66, 3.74)	
>5 talc-years	9	18	1.85 (1.21, 2.80)	4	0.44 (0.10, 1.90)	13	1.33 (0.77, 2.31)	9	0.87 (0.32, 2.38)		12	1.66 (0.96, 2.88)	15	2.28 (1.23, 4.26)	
P trend			0.003		0.24		0.34		0.88	0.06		0.09		0.005	0.28
Postmenopausal	N = 1,168	N = 686		N = 44		N = 150		N = 58			N = 75		N = 39		
No use	70	63	1.00	77	1.00	63	1.00	69	1.00	0.29	65	1.00	77	1.00	0.43
Any use	30	37	1.36 (1.10, 1.67)	23	0.70 (0.34, 1.46)	37	1.36 (0.94, 1.97)	31	1.10 (0.61, 1.99)		35	1.15 (0.69, 1.91)	23	0.80 (0.37, 1.75)	
No genital use	70	64	1.00	77	1.00	63	1.00	69	1.00		65	1.00	77	1.00	
≤5 talc-years	13	15	1.44 (1.07, 1.93)	5	0.34 (0.08, 1.46)	15	1.39 (0.82, 2.33)	14	1.32 (0.58, 2.99)		16	1.40 (0.70, 2.79)	10	1.24 (0.41, 3.77)	
>5–24 talc-years	8	9	1.19 (0.83, 1.71)	14	1.67 (0.66, 4.20)	9	1.15 (0.61, 2.19)	3	0.39 (0.09, 1.69)		8	1.11 (0.45, 2.73)	8	1.03 (0.30, 3.55)	
>24 talc-years	9	12	1.33 (0.96, 1.85)	5	0.45 (0.10, 1.91)	13	1.60 (0.93, 2.77)	14	1.59 (0.70, 3.60)		11	0.99 (0.44, 2.21)	5	0.39 (0.09, 1.76)	
P trend			0.13		0.49		0.12		0.44	0.58		0.91		0.23	0.29

[a]Adjusted for reference age (continuous), study center, study phase (3, 4, 5), parity (nulliparous, 1, 2, ≥2), breastfeeding (never, <4, 4–9, 10–19, >19 months), OC use (never, <23, 23–49, 50–96, >96 months), HT use (premenopausal, postmenopausal never used, postmenopausal used HT), IUD (never, ever), endometriosis or painful periods, personal history of breast cancer, Jewish ethnicity, tubal ligation, and BMI (<22.2, 22.2–24.8, 24.9–28.6, >28.6).

TABLE 5. Associations Between Genital Talc Use (Never/Ever and Quartiles of Talc-years) and Ovarian Cancer by Menopausal Status and Postmenopausal Hormone Therapy Among Restricted Histologic Types

	Premenopausal			Postmenopausal, Never Used HT			Postmenopausal, Ever Used HT		
Genital Talc Use	Controls N (%)	Cases[a] N (%)	Adjusted[b] OR (95% CI)	Controls N (%)	Cases[a] N (%)	Adjusted[b] OR (95% CI)	Controls N (%)	Cases[a] N (%)	Adjusted[b] OR (95% CI)
Never	735 (79)	531 (72)	1.00 (referent)	507 (69)	378 (69)	1.00 (referent)	309 (72)	152 (53)	1.00 (referent)
Ever	197 (21)	211 (28)	1.42 (1.12, 1.81)	230 (31)	173 (31)	1.00 (0.78, 1.28)	122 (28)	133 (47)	2.32 (1.64, 3.27)
No genital use	735 (79)	531 (72)	1.00 (referent)	507 (69)	378 (69)	1.00 (referent)	309 (72)	152 (54)	1.00 (referent)
≤1	70 (8)	47 (6)	0.90 (0.60, 1.37)	40 (6)	36 (7)	1.32 (0.80, 2.17)	28 (7)	28 (10)	2.02 (1.10, 3.70)
>1–5	44 (5)	52 (7)	1.66 (1.06, 2.60)	52 (7)	32 (6)	0.81 (0.50, 1.32)	28 (7)	29 (10)	2.56 (1.40, 4.67)
>5–24	59 (6)	68 (9)	1.54 (1.04, 2.28)	61 (8)	41 (8)	0.86 (0.55, 1.33)	26 (6)	30 (11)	2.18 (1.19, 4.00)
>24	21 (2)	41 (6)	2.33 (1.32, 4.12)	70 (10)	56 (10)	1.00 (0.68, 1.49)	36 (8)	43 (15)	2.57 (1.51, 4.36)
P trend			0.0006			0.88			0.001

[a]Postmenopausal cases are restricted to serous and endometrioid invasive, premenopausal cases additionally include serous and mucinous borderline cases.
[b]Adjusted for reference age (continuous), study center, study phase (3, 4, 5), parity (nulliparous, 1, 2, ≥2), breastfeeding (never, <4, 4–9, 10–19, >19 months), OC use (never, <23, 23–49, 50–96, >96 months), IUD (never, ever), endometriosis or painful periods, personal history of breast cancer, Jewish ethnicity, tubal ligation, and BMI (<22.2, 22.2–24.8, 24.9–28.6, >28.6).

truly exposed cases were also correctly classified (sensitivity 99%). The OR of 1.33 in our study would be nullified if the sensitivity of correctly classified controls fell to 82% or 18% misclassification. Unfortunately, there are no external records to validate talc use reported by study participants to assess whether this degree of misclassification is reasonable. Somewhat analogous to talc and ovarian cancer is alcohol use and breast cancer. Nurses' Health Study investigators examined the latter association both with prospective data collected at baseline and retrospective data obtained by resurveying subjects after diagnosis.[25] They found an (age adjusted) OR for breast cancer of 1.42 associated with 30 or more grams of alcohol/day relative to nondrinkers from the prospective data compared with 1.33 from the retrospective data. This change between two analyses would occur if the sensitivity of controls correctly recalling alcohol use dropped to 91% (or 9% misclassification). This suggests some degree of misclassification in retrospective data but not as great as the 18% required to nullify the association between use of talc on genitals and ovarian cancer risk in our study. No comparable study on talc comparing results from prospective versus retrospective data has been performed. However, several observations are incompatible with the possibility that recall bias explains the association: (1) ORs are generally lower in studies which asked about "ever use" of talc[5,8,11] compared with those that specified regular use,[6,7,9,12,13] whereas higher ORs would be expected if cases are more likely to recall limited ever-use; (2) no association with nongenital talc use; (3) risk varies by histologic type; (4) the association is stronger in premenopausal women who are closer in time to talc use and less likely to have forgotten it; and (5) ORs from recent studies[11,13] are lower than those from earlier ones,[6,7] whereas increasing publicity about the association over time might lead to greater recall bias and higher ORs in more recent studies. Related arguments that cases initiate talc use because of treatment of ovarian cancer or early symptoms of disease also lack merit because we censored exposures 1 year before the date of diagnosis and most talc-users began the habit around age 20—a decade or more before the ovarian cancer diagnosis.

Whether the association is a result of confounding must also be addressed. A 1998 article identified BMI, smoking, and alcohol use as potential correlates of talc use in the general population.[26] In our study, powder users were more likely to be older, from more urban/suburban areas, heavier, asthma sufferers, and regular analgesics users. However, none of these or other Table 2 variables altered the overall association by more than 10%, providing no indication of confounding. Talc use was also greater in African Americans and notably associated with a high, albeit imprecise, OR (and 95% CI) of 5.08 (1.32, 19.6). This finding clearly requires further study.

The observation that talc users, both case and control subjects, were more likely to say they had asthma has not been previously reported. The link between powder use and asthma may not be fully appreciated from Table 2 since women who used talc as a body powder but not to the genital area were classified as nonexposed. Making no body or genital exposure the nonexposed referent group and asthma the outcome, the ORs (and 95% CI) for asthma for body exposure to talc is 1.27 (0.80, 2.03) for cases and 1.02 (0.66, 1.57) for controls. The comparable OR for genital use of talc with or without body use is 1.48 (1.00, 2.18) for cases and 1.45 (1.00, 2.10) for controls. Sixty of 85 cases (70%) with asthma and 57 of 89 (64%) controls reported that talc use predated asthma onset. Although chance must be considered a possible explanation for this novel finding, talc is a cause of occupational asthma[27] and respiratory distress has been reported in infants after talc was accidentally inhaled.[28] That asthma may be associated with use of talc is important not only because of health

consequences on its own but also because it may shed light on biologic mechanisms potentially relevant to the talc and ovarian cancer association.

Although we found no evidence of confounding, we did find several examples of effect modification of the association between talc and epithelial ovarian cancer. Overall, the association was greater in women with no personal history of breast cancer, those who had a tubal ligation or hysterectomy, in premenopausal women, and postmenopausal women who had used HT. Among these factors, perhaps the most important is effect modification of the association by menopausal status and menopausal HT.

Apparent lack of an elevated risk for epithelial ovarian cancer from talc use in postmenopausal women without HT use has not been reported previously. Explanations might include that there is no association with talc use in the absence of endogenous or exogenous estrogen, fading memory of past exposures, women who will develop ovarian cancer from talc use leave the at risk pool before they reach menopause, or more complex interactions with multiple risk factors and gene–environment interactions. Of possible relevance, Moorman et al.[29] observed that reproductive events that clearly affect risk in premenopausal women may not affect risk to the same degree in postmenopausal women. Whatever the explanation, our observation challenges the relevance of the WHI study to the ovarian cancer/talc association since only postmenopausal women were enrolled in WHI and HT use was examined only as a confounder, not an effect modifier.[16] Further study will be necessary to clarify the role that talc may play in postmenopausal women who did not use HT with a focus on those factors that may increase endogenous estrogen, such as greater BMI.

That the association is more apparent in premenopausal women and in postmenopausal women who used hormonal therapy suggests that estrogen plays a role in the association. In talc inhalation studies conducted by the National Toxicology Program, only female rats developed lung tumors.[30] Literature on airway inflammation from particulates is also relevant. Citing evidence that asthma may be exacerbated during pregnancy, Zhang et al.[31] postulated this may be due to an effect of estrogen on macrophage activity and inflammatory response to particulates normally considered inert, like titanium dioxide (TiO_2). Their in-vivo studies demonstrated that macrophages from pregnant mice transplanted to nonpregnant recipients conferred an inflammatory phenotype in response to TiO_2. Such studies should be repeated with talc, another particulate considered "inert."

An exploratory analysis of other potential effect modifiers led to several other observations that may have biologic relevance. The overall associations and dose–responses were "stronger" for premenopausal women who had a greater BMI, had breastfed, were not current smokers, and consumed alcohol (Fig. 3). Due to the large number of associations tested, chance must be the first explanation considered. However, a common denominator could be prolactin since its levels are higher in women who have greater BMI,[32] breastfed,[33] do not currently smoke,[34] consume alcohol,[35] and are postmenopausal and use HT.[36] Like estrogen, prolactin may have multiple effects on immune cells, especially monocytes and macrophages[37] whose role in scavenging talc in tissue is described.[38] These observations provide a framework for talc carcinogenicity in EOC involving chronic inflammation.[9]

Biologic credibility of the talc/EOC association is enhanced by persuasive evidence that inert particles the size of talc, present in the vagina, can migrate to the upper genital tract. In a technique called hysterosalpingoscintigraphy, technetium-labeled albumen microspheres are placed in the vagina and their migration to the upper tract was confirmed using serial scintograms.[39] The microspheres are 5 to 40 μm in diameter—a range which includes the size of sperm and talc. Migration from the vagina is the obvious explanation for why talc can be found in diseased (and some normal) ovaries.[3] Unfortunately, no epidemiologic study of epithelial ovarian cancer and talc has taken the opportunity to determine whether talc can actually be found in tissues removed at surgery and correlated with exposure to talc. A clue to talc's presence is birefringent particles found when slides are examined under polarized-light microscopy. Although confirmation that the material is actually talc requires scanning electron microscopy and X-ray dispersion spectroscopy, presence of birefringence is a practical screening technique as illustrated by a case report of a woman with ovarian cancer and long-term talc use who had talc in her pelvic lymph nodes first suggested by birefringence.[40]

There are inherent limitations quantifying a dose–response due to a lack of metrics for how much talc is in an "application," how much enters the vagina, and how much reaches the upper genital tract where, presumably, any deleterious effect is mediated. This may account for the failure to identify a dose–response in many papers on talc and ovarian cancer. Our 1999 study[7] suggested that adjusting total applications by whether the genital tract was "open" (i.e., excluding use after a tubal ligation or hysterectomy and examining use during times when ovulation was occurring) yielded significant dose–responses. Mills et al.[10] found a dose–response by frequency of use. Wu et al.,[12] looking at all types of body use, found a dose–response with estimated applications. Merritt et al.[11] reported a significant trend in risk for invasive serous ovarian cancer with years of talc use. The recent OCAC analysis reported no trend with increasing lifetime applications *when restricted to talc users*.[19] However, an increase in risk with increasing applications was found for nonmucinous epithelial ovarian cancer *when nonusers were included*. Virtually all papers that have looked at dose–response for talc and epithelial ovarian cancer risk have included nonusers in the trend analysis. In our article, we calculated talc-years and showed that, overall, there is a significant trend for epithelial ovarian cancer risk and talc-years when nonusers are included, and the

trend is even more apparent in premenopausal women with certain epithelial ovarian cancer subtypes.

In summary, this study on talc and epithelial ovarian cancer has contributed the following perspectives, some new, regarding this association:

(1) Overall, there is an association between genital talc use and EOC and a significant trend with increasing "talc-years" of use.
(2) Among many epidemiologic variables, no confounders for the association were identified.
(3) Talc users, both cases and controls, were more likely to report a medical history of asthma.
(4) The talc/epithelial ovarian cancer association was largely confined to premenopausal women and postmenopausal women who used HT. Other potential effect modifiers in premenopausal women included BMI, breastfeeding, current smoking, or alcohol use. These observations may suggest a role for estrogen and/or prolactin, both known to affect macrophage function and inflammatory response.
(5) Histologic subtypes of epithelial ovarian cancer more likely to be associated with talc include serous and mucinous borderline tumors and invasive serous and endometrioid tumors.
(6) For epithelial ovarian cancer categories based on certain effect modifiers or histologic subtypes, stronger overall associations and dose–responses were observed.
(7) The association may be stronger in African Americans.

An editorial[17] accompanying the WHI study[16] noted that "several case–control studies have reported associations between talc use and ovarian cancer risk" and "no epidemiologic studies have demonstrated a dose–response" (page 2). We believe these appraisals understate the epidemiologic evidence. There have been dozens of case–control studies and several have, in fact, found a dose–response. The editorial further notes that "it does not seem likely that additional conventional epidemiologic studies will strengthen the evidence for or against talc carcinogenicity" (page 2). We believe the observations made here present a good case for talc carcinogenicity and that reanalyses of existing data from already published studies might provide confirmatory evidence. To encourage consolidation of data, we have provided a copy of the "raw" and derived variables examined in our study to NCI dbGaP (available here: http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001034.v1.p1) as well as the SAS and Stata programs used in this analysis (eAppendix 1; http://links.lww.com/EDE/B2).

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Exhibit 50

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Genital powder use and risk of ovarian cancer: a pooled analysis of 8,525 cases and 9,859 controls

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Abstract

Genital powder use has been associated with risk of epithelial ovarian cancer in some, but not all, epidemiologic investigations, possibly reflecting the carcinogenic effects of talc particles found in most of these products. Whether risk increases with number of genital-powder applications and for all histologic types of ovarian cancer also remains uncertain. Therefore, we estimated the association between self-reported genital powder use and epithelial ovarian cancer risk in eight population-based case-control studies. Individual data from each study was collected and harmonized. Lifetime number of genital-powder applications was estimated from duration and frequency of use. Pooled odds ratios were calculated using conditional logistic regression matched on study and age and adjusted for potential confounders. Subtype-specific risks were estimated according to tumor behavior and histology. 8,525 cases and 9,859 controls were included in the analyses. Genital powder use was associated with a modest increased risk of epithelial ovarian cancer (odds ratio 1.24, 95% confidence interval 1.15–1.33) relative to women who never used powder. Risk was elevated for invasive serous (1.20, 1.09–1.32), endometrioid (1.22, 1.04–1.43), and clear cell (1.24, 1.01–1.52) tumors, and for borderline serous tumors (1.46, 1.24–1.72). Among genital powder users, we observed no significant trend (p=0.17) in risk with increasing number of lifetime applications (assessed in quartiles). We noted no increase in risk among women who only reported non-genital powder use. In summary, genital powder use is a modifiable exposure associated with small-to-moderate increases in risk of most histologic subtypes of epithelial ovarian cancer.

Keywords

ovarian cancer; powder; talc; epidemiology

INTRODUCTION

Powders that are commonly applied either directly to the genital, perineal, or rectal area after bathing or indirectly to underwear, sanitary napkins, tampons, or stored contraceptive devices may contain talc because of its softness, absorbency, and lack of clumpiness (1). However, the presence of talc in commercially available powder formulations has varied over time, even within particular brands of products, limiting the ability of most epidemiologic studies to measure genital talc exposure accurately. Despite this, genital powder use, but not use on other parts of the body, has been linked to increased risk of ovarian cancer, suggesting that powder particles ascending the genital tract may predispose to ovarian cancer development (2–4). Meta-analyses of observational studies show 33–35% increased risk of ovarian cancer among women who have used genital powders (1, 4, 5), but evidence for a dose-response relationship has been inconsistent. Though dose response was not addressed in previous meta-analyses(1, 4, 5) some individual studies have reported significant dose-response (4, 6–10) while others have not (9, 11–15).

Epidemiologic and biologic studies show differences in risk-factor profiles and molecular characteristics between ovarian cancer subtypes defined by histology (serous, endometrioid, mucinous, clear cell) and behavior (borderline, invasive) (16). For instance, serous tumors are characterized by p53 mutations, while mucinous tumors have a high prevalence of KRAS mutations (17) and are not generally associated with reproductive risk factors (16, 18). Since most early studies of powder use and ovarian cancer did not include analysis by

histologic subgroups (3, 6, 11, 19–21), histology-specific estimates were not available from these studies for meta-analysis. Most (2, 4, 8, 9, 22), but not all (10, 14, 15, 23), epidemiologic studies of genital powder use and risk of ovarian cancer that have evaluated histologic subgroups have found the association to be strongest for serous invasive tumors. Such tumors comprise the most common variety of ovarian cancer and few previous studies have had sufficient statistical power to evaluate the association between genital powder use and risk of other histologic subtypes. In the present study, we evaluated associations between genital powder use and risk of ovarian cancer overall, by invasiveness and by histologic type in a pooled analysis of eight population-based case-control studies with relevant data from the Ovarian Cancer Association Consortium (OCAC), a consortium founded in 2005 to validate promising genetic associations in epidemiologic studies of ovarian cancer.

MATERIALS AND METHODS

Participating studies

Studies participating in the OCAC consortium as of April 2010 that collected data on powder use were included. Each study was approved by an institutional ethics committee and all participants provided informed consent. Detailed description of the OCAC consortium is available elsewhere (24). Characteristics of the eight case-control studies contributing data to this analysis are presented in Table 1. Six studies were conducted in the USA (DOV (14), HAW (25), HOP (26), NCO (27), NEC (4), USC (28)) one study in Australia (AUS (7)) and one study in Canada (SON (15)). Overall, our analyses included 8,525 cases of ovarian, fallopian tube or peritoneal cancer and 9,859 controls. Five studies previously reported on powder use (AUS (7), DOV (14), NCO (27), NEC (4), SON (15),) three of which provided data for this analysis that had not been included in their previous powder-related publication (DOV, NEC, AUS). The remaining three studies have not previously published their genital powder-use data (HAW, HOP, USC).

Exposure and covariate data

Data collected from participants regarding genital powder use varied between studies. Harmonized analytic exposure variables were developed by comparing questionnaires between the eight participating studies. The majority of the studies have obtained information on duration and frequency of powder use, age at first powder use, use by sexual partners, and non-genital use (Table 1). We defined genital powder use as any type of powder (talc, baby, deodorizing, cornstarch, or unspecified/unknown) applied directly or indirectly (by application to sanitary pads, tampons, underwear) to the genital, perineal, or rectal area. Since study specific powder questions included varying degrees of detail regarding type and method of application, genital powder definitions differ between studies. Criteria for regular genital powder use varied between studies from “ever use” (AUS) to “one year or longer” (DOV); the specific wording for this question is provided in Table 1. Use of body powders on sites other than the genital area was defined as non-genital powder use. Women who reported both genital and non-genital powder use were classified as genital users. Two studies (DOV, SON) did not collect data on non-genital use and therefore women assigned to “no powder use” for these studies could have a history of non-genital powder exposure. Extensive information on known and suspected risk factors for ovarian cancer was collected in each study, including oral contraceptive (OC) use, parity, tubal ligation history, body mass index (BMI), race, and ethnicity.

Statistical methods

Participants missing case/control status (n=17) or tumor histology (n=19) were excluded from the analysis. We also excluded 1,119 participants who answered “do not know” or

were missing data on genital powder use; most of these were from the NCO study which did not include genital powder questions for the first 720 participants. Furthermore, we excluded participants missing tubal ligation (n=55), OC duration (n=100), parity (n=3), or height or weight (BMI) (n=179). To examine differences in characteristics between cases and controls, we evaluated two-sample t-statistics (age, BMI) and chi square statistics (OC use, nulliparity, tubal ligation, race/ethnicity, powder use).

Study-specific odds ratios (ORs) and 95% confidence intervals (CI) were estimated using unconditional logistic regression and were summarized by forest plots, including study heterogeneity based on Cochran's Q statistic. As no significant heterogeneity was observed between studies, we calculated pooled ORs and 95% CIs across the studies using conditional logistic regression matched on 5-year age groups and study. All analyses were adjusted for potential confounders: age (continuous), duration of oral contraceptive (OC) use (never use, use <2yrs, 2–<5yrs, 5–<10yrs, ≥10yrs), parity (0, 1, 2, 3, ≥4 children), tubal ligation history, BMI (quartiles based on distribution in controls), and race/ethnicity (non-Hispanic white, Hispanic white, black, Asian, other). Family history of breast or ovarian cancer were also considered as covariates but were not included in the final model.

Subtype-specific estimates were calculated for subgroups of ovarian cancer defined by behavior (invasive, borderline) and histology (serous, mucinous, endometrioid, clear cell) by comparing each case group to all controls. As borderline endometrioid and clear cell tumors are rare, we did not have sufficient numbers to evaluate those types separately.

In order to measure cumulative dose of genital-powder use, we estimated lifetime number of powder applications by multiplying total months of use by frequency of use per month, for all direct and indirect genital-powder applications. Women who reported multiple types of genital powder exposure (on underwear, on sanitary napkins or pads, directly to genital area) during the same time period were assigned the number of genital powder applications equal to the most commonly used type rather than the sum of applications across all types of genital powder exposure. We reasoned that contemporaneous powder applications were unlikely to be independent events and therefore should not be treated cumulatively.. Analyses of estimated lifetime number of applications excluded participants in the HOP study as data on age and frequency of use were not collected (n=2,224); genital powder users missing information on duration or frequency of use were omitted in the remaining studies (n=394). Never regular users of genital powders and women who only reported non-genital use were coded as having zero lifetime genital powder applications and comprised the reference group for this analysis. Categories were determined based on age-specific quartile cutpoints in controls (25th, 50th and 75th percentile cutpoints are 612, 1,872, and 5,400 for participants < 40 years old; 612, 2,160, and 7,200 for 41–50 years; 720, 3,600, and 10,800 for 51–60 years; 1,440, 5,760, and 14,440 for 61–70; 840, 7,200, and 18,000 for > 70 years). Trends were evaluated based on the median lifetime number of genital-powder applications for controls in each age-specific quartile using the Wald statistic and were performed both including and excluding never users of genital powders.

We estimated the association between genital powder use and ovarian cancer risk within strata to evaluate potential modification of effect defined using a cutpoint BMI of 30 based on the World Health Organization's definition of obesity, endometriosis, parity, tubal ligation/hysterectomy, and menopausal status. We used likelihood-ratio statistics comparing models with and without interaction terms to determine statistically significant interactions. To estimate calendar year of first use, we subtracted the years since first use (age at study entry minus age at first genital powder use) from median calendar year of the participant's study. All analyses were performed in SAS v9.2 (SAS, Cary, NC) and Stata v9.2 (StataCorp,

College Station, TX). All p-values are two-sided. Analyses have been independently verified by two separate study groups (HAW and NCO).

RESULTS

This pooled analysis of eight case-control studies included 9,859 controls and 8,525 ovarian cancer cases. Genital powder use was reported by 2,511 (25%) of the controls and 2,600 (31%) of the cases, while powder use only on other (non-genital) parts of the body was reported by 1,533 (16%) of the controls and 1,282 (15%) of the cases (Table 2). The prevalence of genital powder use in controls varied widely between study sites, highest in AUS (45%) and lowest in HAW (15%, Table 3).

In the pooled analysis, ever regular use of genital powder was associated with a modest increase in risk of ovarian cancer (OR=1.24, 95% CI=1.15–1.33, Table 3) relative to women who reported no powder use (AUS, HAW, HOP, NCO, NEC, USC) or no genital powder use (DOV, SON). We observed no heterogeneity in the risk associated with genital powder use between studies regardless of the reference group (p=0.61, Figure 1). Results were similar for genital powder users compared to a combined reference group including never users and women whose use of powder was exclusively non-genital (covariate-adjusted OR=1.25, 95% CI= 1.16–1.34; data not shown), reflecting the absence of an association between powder use on other parts of the body with ovarian cancer risk (Table 3).

Genital powder use was associated with a similar increased risk of borderline and invasive ovarian cancer overall (Table 4). For borderline tumors, the association was stronger for the serous subtype (OR=1.46, 95% CI=1.24–1.72; Table 4) and non-significant for the mucinous subtype. For invasive ovarian cancer, we observed small increases in risk of serous (OR=1.20, 95% CI=1.09–1.32), endometrioid (OR=1.22, 95% CI=1.04–1.43), and clear cell (OR=1.24, 95% CI=1.01–1.52) cancer but no significant increase in risk of mucinous cancer (OR=1.09, 95% CI= 0.84–1.42). Similarly, we observed no significant increase in risk when borderline and invasive mucinous tumors were considered together (data not shown). Risk associated with genital powder use was consistent across studies for borderline and invasive tumors as well as invasive serous, endometrioid, and clear cell subtypes (p for heterogeneity >0.1; Figures 2 a,b,c,d,e), but not for mucinous tumors (p=0.08; Figure 2f). Genital powder use was associated with increased risk of invasive mucinous tumors in SON, HOP (significantly), and USC (non-significantly) while in the remaining studies (HAW, NCO, AUS, DOV, and NEC) genital powder use was non-significantly associated with reduced risk.

We evaluated cumulative genital-powder exposure as a composite variable of frequency and duration of use. We observed similar increased risks of all non-mucinous subtypes of epithelial ovarian cancer combined across quartiles of genital powder compared to non-use: OR_{Q1}=1.18, 95% CI=1.02–1.36, OR_{Q2}=1.22, 95% CI=1.06–1.41, OR_{Q3}=1.22, 95% CI=1.06–1.40, OR_{Q4}= 1.37, 95% CI=1.19–1.58 (Table 5). Although a significant increase in risk with an increasing number of genital powder applications was found for non-mucinous epithelial ovarian cancer when non-users were included in the analysis (p-trend<0.0001), no trend in cumulative use was evident in analyses restricted to ever-users of genital powder (p-trend=0.17; Table 5). Taken together, these observations suggest that the significant trend test largely reflects the comparison of ever regular use to never use. Since tubal ligation or hysterectomy would block the transport of powder through the genital tract to the ovaries, we performed a sensitivity analysis excluding women who started genital powder use after these procedures. We observed similar associations when we excluded the 65 cases and 79 controls who started genital powder use for the first time after surgery (OR_{Q1}=1.19, 95% CI=1.03–1.38, OR_{Q2}=1.19, 95% CI=1.03–1.38, OR_{Q3}=1.21, 95% CI=1.04–1.39, OR_{Q4}=

1.36, 95% CI=1.18–1.57). For studies that collected data on timing of powder use and tubal ligation/hysterectomy, we were able to identify timing of genital powder exposure in relation to surgery based on age of powder use and age at surgery. Restricting our exposure to genital powder applications that occurred before tubal ligation or hysterectomy made no substantive difference in the results.

The association between any genital-powder use and ovarian cancer risk was stronger among women with BMI < 30 kg/m^2 (OR=1.28, 95% CI=1.17–1.39) than for women with BMI > 30 (OR=1.14, 95% CI=0.98–1.32, p-interaction=0.01). We observed no significant interactions between genital powder use and parity, reported history of endometriosis, tubal ligation/hysterectomy, or menopausal status (all p-interaction > 0.1). The association between genital powder use and ovarian cancer risk was similar for women who started use between 1952 and 1961 (OR=1.36, 95% CI=1.19–1.56), between 1962 and 1972 (OR=1.27, 95% CI=1.11–1.46), and after 1972 (OR=1.31, 95% CI=1.15–1.51). However, we observed an attenuated association for women who started genital powder use before 1952 (OR=1.08, 95% CI=0.93–1.25).

DISCUSSION

This pooled analysis of eight case-control studies suggests that genital powder use is associated with a modest 20–30% increase in risk of developing epithelial ovarian cancer, including serous, endometrioid, and clear cell tumors, but is less relevant to invasive mucinous tumors. Our findings are consistent with and extend the findings of three meta-analyses that have reported an increased risk of epithelial ovarian cancer with genital-powder use (1, 4, 5) by including dose response and histology specific analyses. Our estimate of the overall association between genital powder use and ovarian cancer risk was slightly attenuated compared to previous estimates from meta-analyses. Possible reasons for the difference include the lack of restriction to published results, data harmonization between studies that allowed similar definitions for the exposure and covariates, and chance. Based on the consistency in the epidemiologic literature on talc-based powder and ovarian cancer risk, the International Agency for Research on Cancer (IARC) classified talc-based body powder as a class 2b carcinogen “possibly carcinogenic to human beings” (29).

The biologic plausibility for the observed association between genital-powder use and ovarian cancer risk has been challenged because evidence for dose-response has been inconsistent (2, 4, 5, 9, 10, 15, 22). The lack of significant dose-response may reflect the difficulty inherent in accurate recollection of specific details of frequency and duration of genital-powder use. Also, because not all powder products contain talc, various products may differ in their potential carcinogenic effects. Alternatively, the association between genital-powder exposure and ovarian cancer risk may not be linear and a modest exposure may be sufficient to increase cancer risk. Talc-containing powders are hypothesized to promote cancer development by ascending the female genital tract and interacting directly with the ovarian surface epithelium, leading to local inflammation characterized by increased rates of cell division, DNA repair, oxidative stress, and elevated inflammatory cytokines (13). Particles in solution easily ascend the genital tract (30, 31). Our finding of slightly attenuated associations following exclusion of women with powder exposure after tubal ligation or hysterectomy are not supportive of this hypothesis, but risk estimates in this subgroup analysis may have randomly differed from those including all women because of the reduction in sample size. Talc particles have been observed in the ovaries of humans (32) and in rodent models (33, 34), but little is known about the biologic effects of genital powder use.

In the current analyses of the various histological subtypes of ovarian cancer, we confirmed previous reports of increased risk of serous invasive tumors with genital-powder use (2, 4, 8, 9, 22). We also observed significantly increased risk of both endometrioid and clear cell invasive ovarian tumors with use of genital powder, and this finding was consistent across studies. It has been suggested that both endometrioid and clear cell ovarian tumors may originate from ectopic uterine endometrium (endometriosis) implanted on the ovary (17). In contrast, we observed no significant associations between genital powder use and either borderline or invasive mucinous ovarian cancer. The lack of a significant association for mucinous tumors may be due to the relatively small number of these tumors or could be an indication that powder exposure is not relevant to the pathogenesis of this histologic type. Studies have noted that ovarian cancer risk factors and molecular characteristics differ for mucinous tumors (16–18, 23, 35–39).

Limitations of our pooled analysis include differences in the wording of questions about genital powder use between studies and the retrospective nature of the exposure ascertainment. Women who were classified as genital-powder users varied from “ever” use (AUS) or “ever regular” use (SON) to powder use for at least six months (HAW, HOP, NCO, NEC, USC) or at least one year (DOV). Differences in genital powder questions result in varying levels of misclassification of true genital powder exposure. However, since exposure definitions are the same for cases and controls within each study, misclassification genital powder exposure due to the question wording would be non-differential, leading to an underestimate of the true association for any given study. These studies were retrospective in nature and therefore potentially susceptible to bias if cases were more likely to report genital-powder use than controls. Although non-genital powder use was not associated with ovarian cancer risk, it is nevertheless possible that any overreporting of powder use by cases might have been limited to reporting of genital powder. Our analyses were also limited by missing data on genital powder use; however, missingness was not associated with the distribution of any of the ovarian cancer risk factors examined and was thus not likely to bias our results. Strengths of our analysis include a large sample size and pooled analysis of individual data, allowing evaluation of the association of genital powder use with less common histologic subgroups of ovarian cancer, careful harmonization of the data based on comparison of study questionnaires, the use of a composite variable combining duration and frequency to assess dose-response relationships.

In conclusion, our large pooled analysis of case-control studies shows a small-to-moderate (20–30%) increased risk of ovarian cancer with genital-powder use, most clearly pertaining to non-mucinous epithelial ovarian tumors. More work is needed to understand how genital powders may exert a carcinogenic effect, and which constituents (e.g. talc) may be involved. Since there are few modifiable risk factors for ovarian cancer, avoidance of genital powders may be a possible strategy to reduce ovarian cancer incidence.

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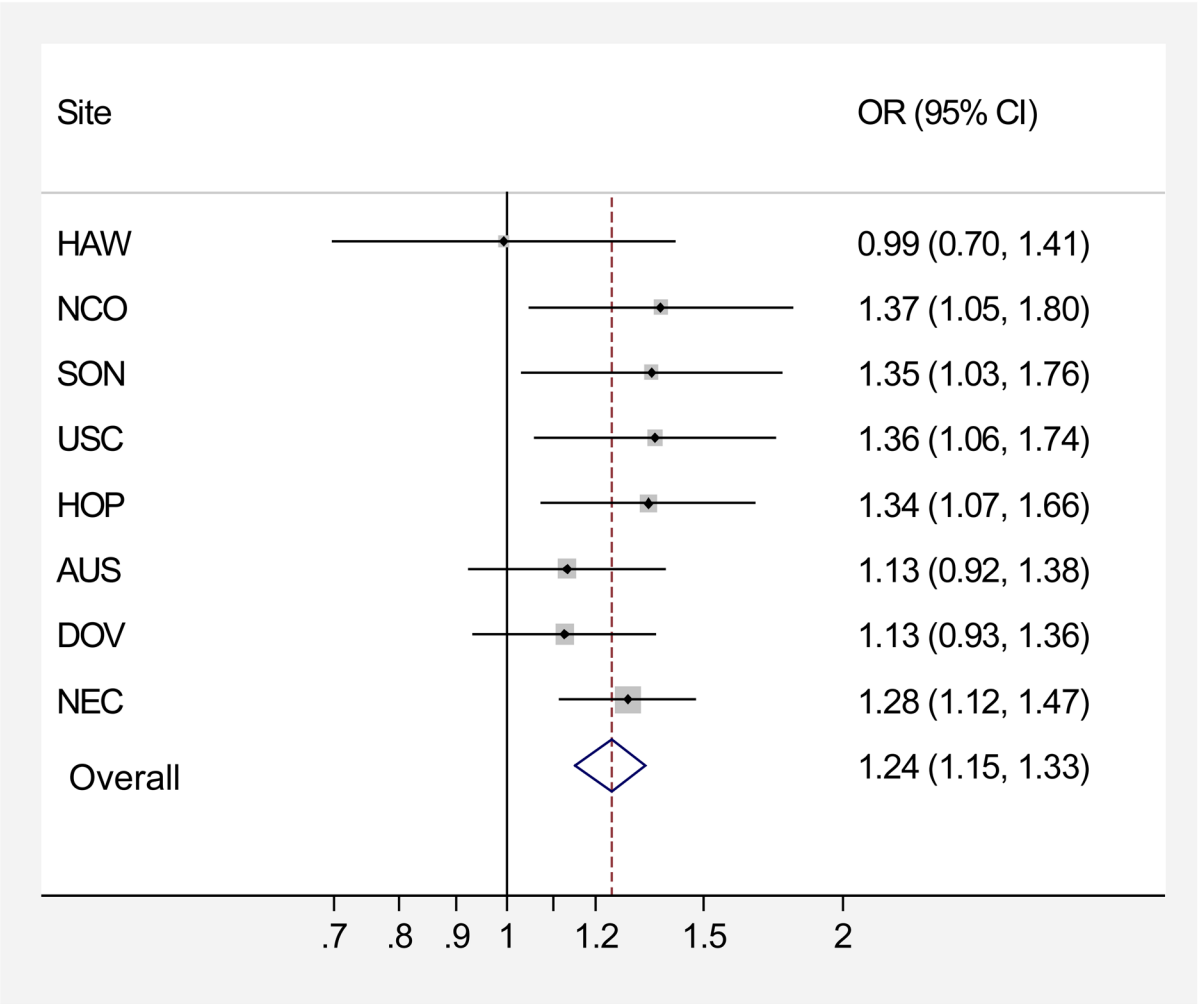
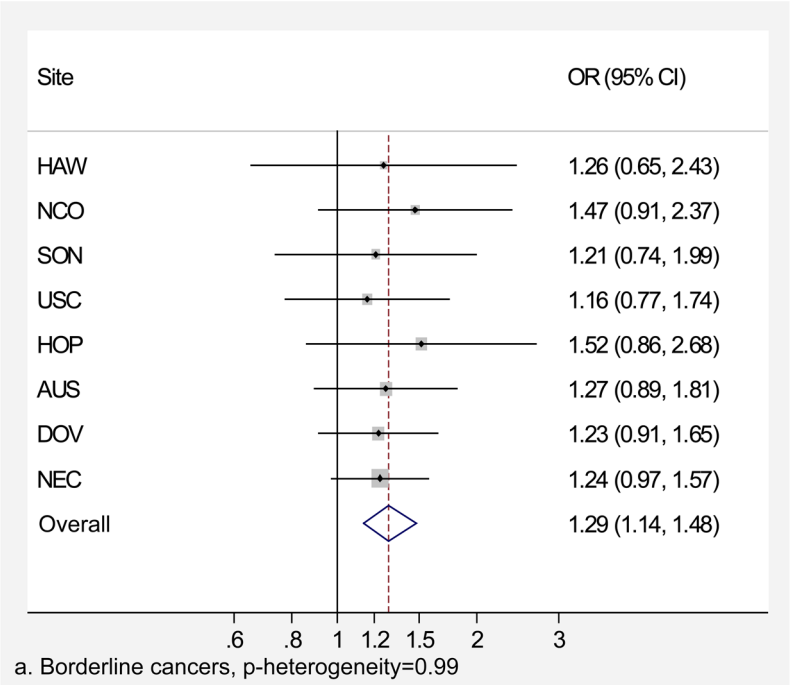
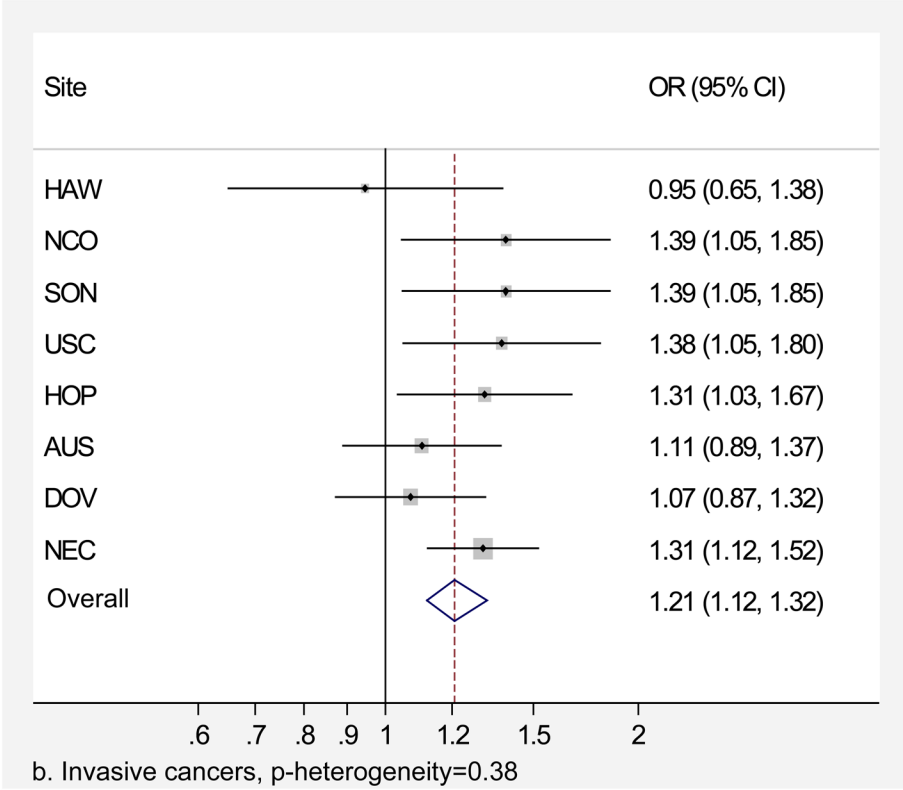


Figure 1.

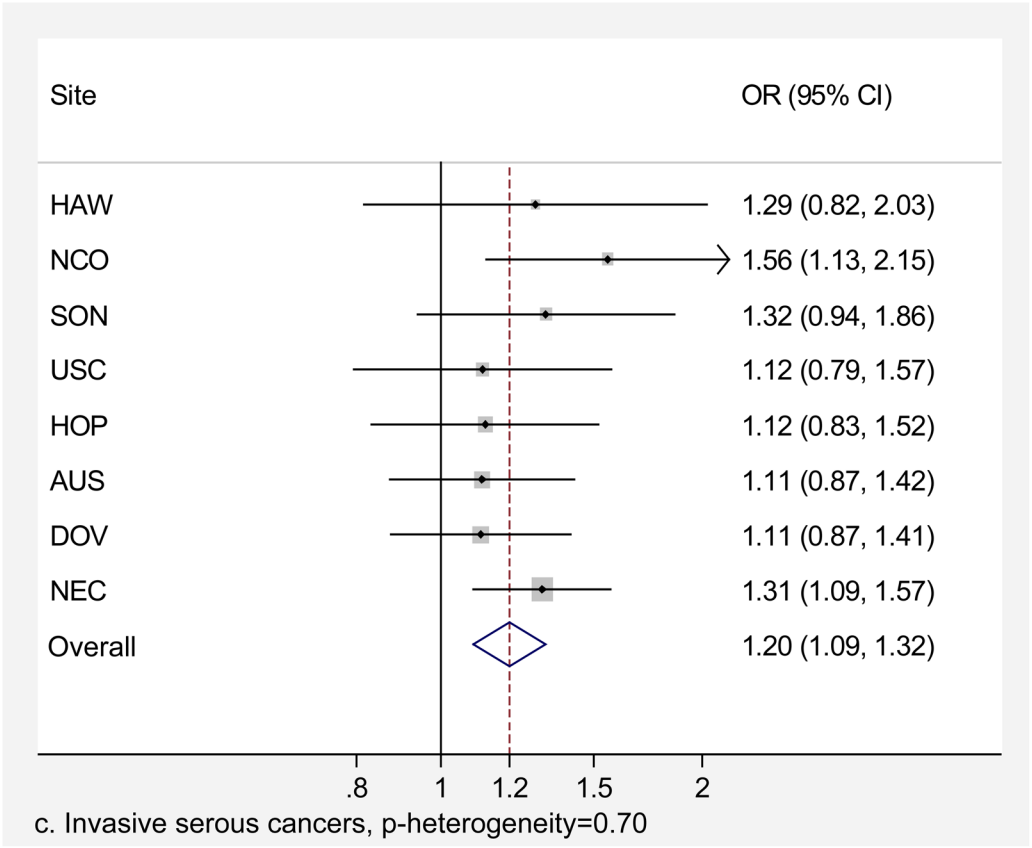
Association between genital powder use and ovarian cancer risk in eight studies, p-heterogeneity=0.61. Adjusted for age (continuous), oral contraceptive duration (never use, <2yrs, 2–<5yrs, 5–<10yrs, >=10yrs), parity (0, 1, 2, 3, 4+ children), tubal ligation history, BMI (quartiles), race/ethnicity (non-Hispanic white, Hispanic white, black, Asian, other). Studies listed in decreasing order of effect size standard error (funnel plot). No evidence of heterogeneity based on Conchran's Q statistic (p=0.61). AUS=Australian Cancer Study, DOV=Diseases of the Ovary and their Evaluation Study, HAW=Hawaii Ovarian Cancer Study, HOP=Hormones and Ovarian Cancer Prediction Study, NCO=North Carolina Ovarian Cancer Study, NEC=New England Case-Control Study of Ovarian Cancer, SON=Southern Ontario Ovarian Cancer Study



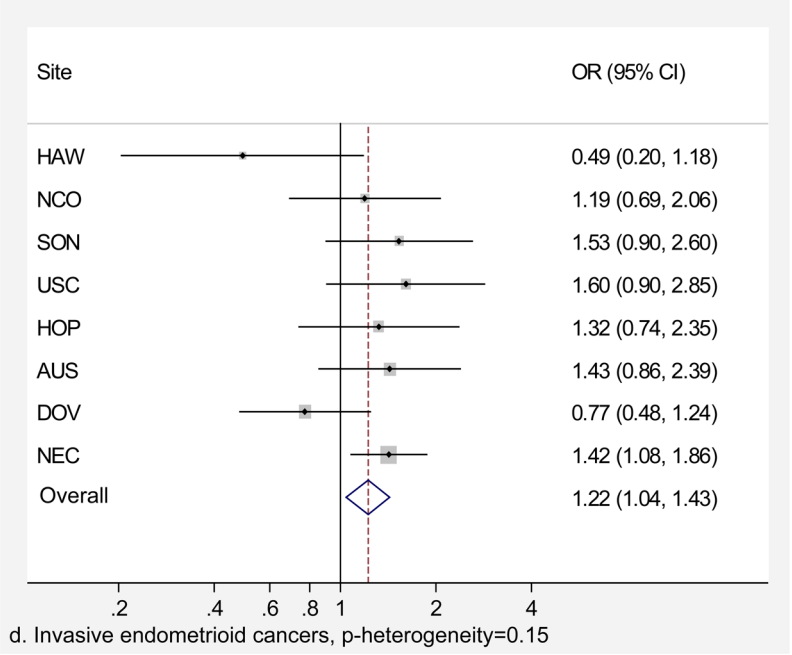
a. Borderline cancers, p-heterogeneity=0.99



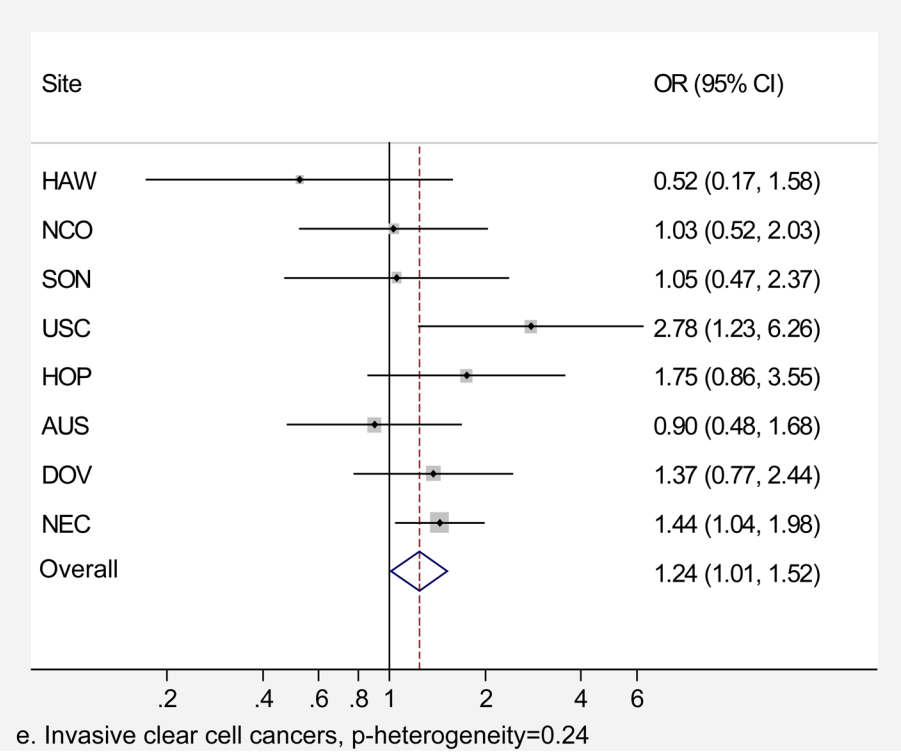
b. Invasive cancers, p-heterogeneity=0.38



c. Invasive serous cancers, p-heterogeneity=0.70



d. Invasive endometrioid cancers, p-heterogeneity=0.15



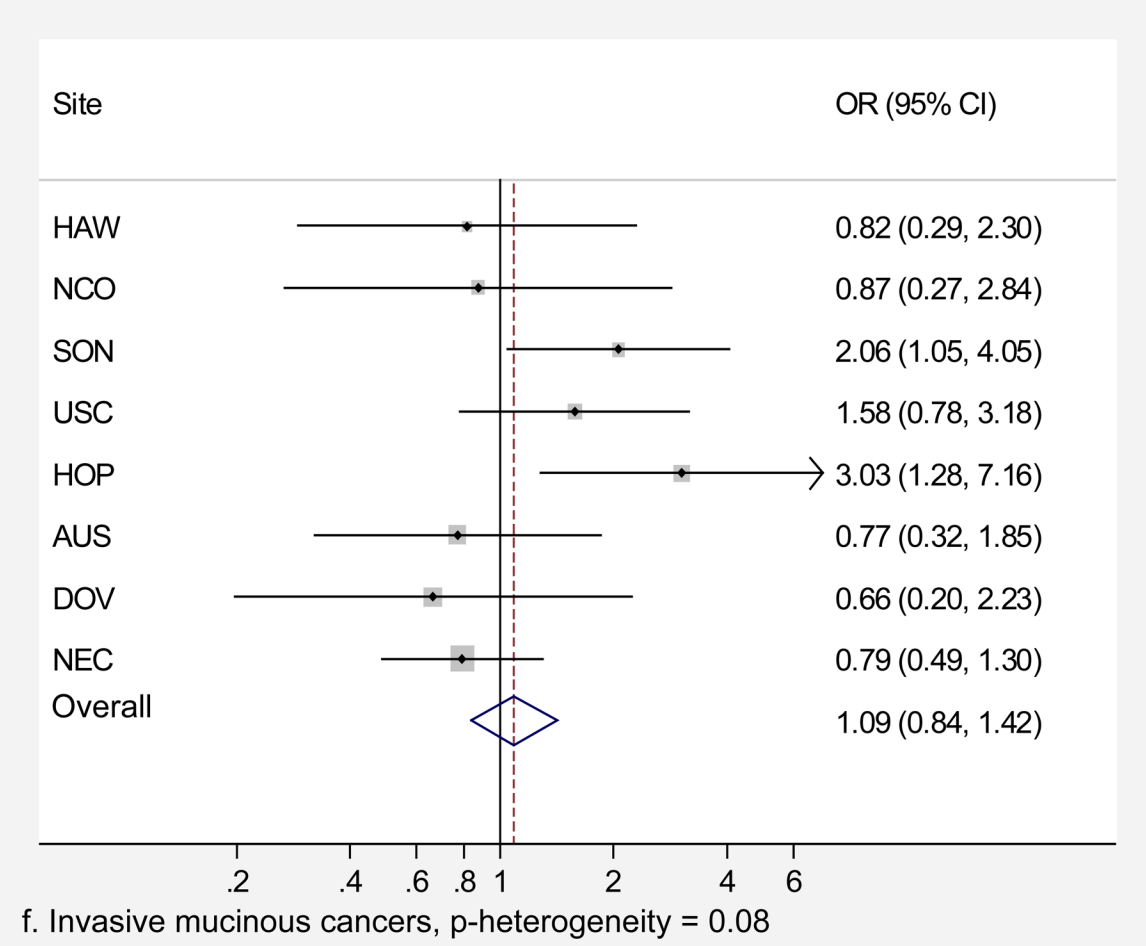


Figure 2.
Association between genital powder use and subgroups of ovarian cancer defined by behavior and histology. Estimates are adjusted for the same covariates as in the model presented in figure 1.

Table 1

Characteristics of eight studies included in the analysis of genital powder use and ovarian cancer

Study*	Diagnosis Years	Controls	Cases	Histology†				Behavior‡		Question used to define genital powder use
				Serous	Mucinous	Endometrioid	Clear cell	Invasive	Borderline	
AUS††	2002–2006	1449	1432	889 (62%)	174 (12%)	132 (9%)	78 (5%)	1158 (81%)	274 (19%)	Have you ever used any sort of powder or talc on your genital area, in your underwear or on a sanitary pad or diaphragm?
DOV††	2002–2009	1841	1565	905 (58%)	186 (12%)	201 (13%)	87 (6%)	1153 (74%)	412 (26%)	Before (reference date) did you ever use any of the following products routinely during one month or more? Powder on sanitary napkins or pads? Vaginal deodorant spray? Before (reference date) did you usually apply any powder to your genital (perineal) area after bathing? We are only interested in times when you did this for at least one year or longer.§
HAW	1993–2008	755	481	222 (46%)	87 (18%)	69 (14%)	47 (10%)	392 (82%)	89 (19%)	Prior to (month/year of diagnosis//) did you ever use talc, baby, or deodorizing powder dusted or sprayed on your body? By regularly I mean at least once a month for 6 months or more. Did you ever use talc, baby or deodorizing powder as a dusting powder to the genital or rectal area? As a dusting powder to sanitary napkins? As a dusting powder to underwear? On a diaphragm or cervical cap?
HOP	2003–2008	1489	735	433 (59%)	53 (7%)	75 (10%)	47 (6%)	568 (88%)	80 (12%)	As an adult and prior to (reference month/year) did you ever use talc or baby powder or deodorizing powder with talc at least once a month for 6 months or more in any of the following ways: As a dusting powder or deodorizing spray more in any of the following ways: As a dusting powder or deodorizing spray to your genital or rectal areas? On your sanitary napkin? On your underwear? On your diaphragm or cervical cap?
NCO††	1999–2008	650	786	489 (62%)	71 (9%)	100 (13%)	65 (8%)	636 (81%)	148 (19%)	Did you ever regularly use cornstarch, talc, baby or deodorizing powders (dusted or sprayed) at least 1 time per month for at least 6 months? If yes, please tell me if you used cornstarch, talc, baby or deodorizing powders in any of the following ways: directly to your genital or rectal areas? Applied to your sanitary napkins or tampons? Applied to birth control devices such as cervical cap or diaphragm? applied to your underwear?
NEC††	1992–2008	2329	2305	1234 (54%)	281 (12%)	352 (15%)	276 (12%)	1659 (77%)	486 (23%)	Did you ever regularly use powder on your body or your underwear (at least once per month for any amount of time)? If yes, did you apply powder directly to your genital or rectal areas? To your sanitary napkins or tampons? To your underwear?
SON††	1989–1992	564	449	254 (57%)	80 (18%)	71 (16%)	29 (6%)	365 (81%)	84 (19%)	Have you ever used sanitary napkins/tampons? If yes, could you tell me over what ages you've used them, for how many years, what percent of periods you've used them for, the usual number you've used for each period,

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Study*	Diagnosis Years	Controls	Cases	Histology†				Behavior‡		Question used to define genital powder use
				Serous	Mucinous	Endometrioid	Clear cell	Invasive	Borderline	
										whether they were deodorant pads/tampons, and if you used talcum powder or starch on them? Have you ever regularly used talcum powder or starch on your vaginal area after showering or bathing?
USC	1993–1997	782	772	396 (52%)	131 (17%)	75 (10%)	32 (4%)	549 (73%)	205 (27%)	Prior to (reference month/year), did you ever regularly use talc, baby, or deodorizing powder dusted or sprayed on your body? By regularly I mean at least once a month for 6 months or more. Did you ever use talc, baby, or deodorizing powder as a dusting powder to the genital or rectal area? as a dusting powder to sanitary napkins? as a dusting powder to underwear? on a diaphragm or cervical cap?

*AUS = Australia Ovarian Cancer Study and Australian Cancer Study (Ovarian Cancer), DOV = Diseases of the Ovary and their Evaluation, HAW = Hawaiian Ovarian Cancer Study, HOP = Hormones and Ovarian Cancer Prediction, NCO = North Carolina Ovarian Cancer Study, NEC = New England Case-Control Study of Ovarian Cancer, SON = Southern Ontario Ovarian Cancer Study, USC = University of Southern California Study of Lifestyle and Women's Health

†Cases listed by histology do not sum because mixed, other, undifferentiated, and unknown are not included.

‡Cases listed by behavior do not sum to the total number of cases because 267 cases are missing behavior information.

§In a separate series of questions, participants were asked about powder use with diaphragm storage. Duration was calculated from ages of use. Information on duration, frequency, and timing of use was only collected on genital/perinal powder use after bathing.

//Controls were asked "Have you ever regularly used…"

**NEC question varied slightly between the three study phases. Between 1992–1997 participants were asked, "As an adult and prior to (reference month/year), did you regularly use talc, baby, or deodorizing powders dusted or sprayed to your body in any of the following ways:". Between 1998–2003, women were asked "Did you regularly apply cornstartch, talc, baby, or deodorizing body powder at least one time per month for six months or longer? If yes, please tell me if you regularly applied cornstarch, talc, baby or deodorizing body powders in any of the following ways:" Between 2003–2008 participants were asked the question listed above.

††These studies previously published on genital powder use and ovarian cancer risk. AUS, DOV, and NEC provided new data to the pooled analyses presented here that were not included in previous publications.

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Table 2

Characteristics of cases and controls included in the pooled analysis *

	Controls (N=9,859) Mean (std) or N (%)	Cases (N=8,525) Mean (std) or N (%)
Age	55 (12)	55 (12)
OC use		
Never	2995 (30)	3411 (40)
Ever	6864 (70)	5114 (60)
Parous		
No	1468 (15)	2196 (26)
Yes	8391 (85)	6329 (74)
Tubal Ligation		
No	7359 (75)	6994 (82)
Yes	2500 (25)	1531 (18)
Body Mass Index	26.5 (6.1)	27.0 (6.6)
Race/Ethnicity		
Non-Hispanic White	8629 (88)	7433 (87)
Hispanic White	197 (2)	214 (3)
Black	273 (3)	268 (3)
Asian	350 (4)	313 (4)
Other †	407 (4)	291 (4)
Powder use ‡		
Never use	5815 (59)	4643 (54)
Non-genital use only	1533 (16)	1282 (15)
Genital use	2511 (25)	2600 (31)

*All characteristics listed except age differed significantly (<0.01) between cases and controls. Cases include both borderline and invasive ovarian cancers.

†There are six cases and three controls missing race/ethnicity information.

‡Categories for non-genital and genital powder use are mutually exclusive.

Table 3

Association between powder use and risk of ovarian cancer (borderline and invasive combined) by study site

Site	Controls (%)(N= 9,859)	Cases (%)(N= 8,525)	Age-adjusted OR (95% CI)*	Multivariate OR (95% CI)*
AUS				
No powder use	305 (21)	300 (21)	1.00	1.00
Non-genital use only	486 (34)	427 (30)	0.85 (0.69–1.05)	0.92 (0.74–1.14)
Genital use	658 (45)	705 (49)	1.04 (0.85–1.26)	1.13 (0.92–1.38)
DOV†				
No powder use	1544 (83)	1293 (83)	1.00	1.00
Genital use	297 (16)	272 (17)	1.14 (0.95–1.37)	1.13 (0.93–1.36)
HAW				
No powder use	489 (65)	326 (68)	1.00	1.00
Non-genital use only	154 (20)	81 (17)	0.79 (0.58–1.07)	0.69 (0.50–0.96)
Genital use	112 (15)	74 (15)	0.99 (0.72–1.37)	0.99 (0.70–1.41)
HOP				
No powder use	989 (66)	439 (60)	1.00	1.00
Non-genital use only	184 (13)	102 (14)	1.23 (0.94–1.61)	1.23 (0.93–1.62)
Genital use	316 (21)	194 (26)	1.37 (1.11–1.69)	1.34 (1.07–1.67)
NCO				
No powder use	391 (60)	469 (60)	1.00	1.00
Non-genital use only	137 (21)	122 (16)	0.75 (0.57–0.99)	0.74 (0.56–0.99)
Genital use	122 (19)	195 (25)	1.33 (1.03–1.74)	1.37 (1.05–1.80)
NEC				
No powder use	1239 (53)	1129 (49)	1.00	1.00
Non-genital use only	454 (19)	421 (18)	1.02 (0.87–1.19)	1.04 (0.88–1.22)
Genital use	636 (27)	755 (33)	1.30 (1.14–1.49)	1.28 (1.12–1.47)
SON†				
No powder use	364 (65)	252 (56)	1.00	1.00
Genital use	200 (35)	197 (44)	1.43 (1.11–1.85)	1.35 (1.03–1.76)
USC				
No powder use	494 (63)	435 (56)	1.00	1.00
Non-genital use only	118 (15)	129 (17)	1.25 (0.94–1.66)	1.14 (0.85–1.52)
Genital use	170 (22)	208 (27)	1.39 (1.10–1.77)	1.36 (1.06–1.74)
Pooled‡				
No powder use	5815 (59)	4643 (54)	1.00	1.00
Non-genital use only	1533 (16)	1282 (15)	0.98 (0.90–1.07)	0.98 (0.89–1.07)
Genital use	2511 (25)	2600 (31)	1.25 (1.16–1.34)	1.24 (1.15–1.33)

*Study-specific estimates were determined using unconditional logistic regression and pooled ORs were estimated using conditional logistic regression conditioned on 5yr age groups and study. Multivariate models are adjusted for age (continuous), oral contraceptive duration (never use, <2yrs, 2–<5yrs, 5–<10yrs, >=10yrs), parity (0,1,2,3,4+ children), tubal ligation history (no, yes), BMI (quartiles), race/ethnicity (non-Hispanic white, Hispanic white, black, Asian, other).

†Information on non-genital powder use was not collected in the SON and DOV study

≠ p-value for heterogeneity between multivariate study specific ORs equal to 0.61; calculated using Conchran's Q statistic test

Table 4

Association between powder use and risk of ovarian cancer by behavior and histology

	Model 1*			Model 2*		
	No powder use	Genital powder use		No genital powder use	Genital powder use	
	n (%)	n (%)	OR (95% CI)†	n (%)	n (%)	OR (95% CI)†
Controls	5815 (59)	2511 (25)		7348 (75)	2511 (25)	
All borderline cases	1035 (58)	504 (28)	1.29 (1.14–1.48)	1247 (72)	504 (28)	1.30 (1.15–1.47)
Serous	567 (57)	300 (30)	1.46 (1.24–1.72)	700 (70)	300 (30)	1.45 (1.24–1.69)
Mucinous	409 (60)	184 (27)	1.17 (0.96–1.42)	502 (73)	184 (27)	1.19 (0.98–1.43)
All invasive cases	3470 (54)	2009 (31)	1.21 (1.12–1.32)	4471 (69)	2009 (31)	1.23 (1.14–1.32)
Serous	1952 (53)	1197 (32)	1.20 (1.09–1.32)	2519 (68)	1197 (32)	1.24 (1.13–1.35)
Mucinous	206 (57)	94 (26)	1.09 (0.84–1.42)	269 (74)	94 (26)	1.06 (0.82–1.36)
Endometrioid	568 (55)	304 (30)	1.22 (1.04–1.43)	723 (70)	304 (30)	1.20 (1.03–1.40)
Clear Cell	327 (54)	187 (31)	1.24 (1.01–1.52)	420 (69)	187 (31)	1.26 (1.04–1.52)

*In model 1, the reference group is restricted to women with no powder use except for the DOV and SON studies as these did not collect data on non-genital powder use. The number of cases who reported non-genital powder use was 212 (13%) of all borderline cases, 133 (13%) serous borderline, 93 (14%) mucinous borderline, 1001 (15%) of all invasive, 567 (15%) serous invasive, 63 (17%) mucinous invasive, 155 (15%) endometrioid invasive, 93 (15%) clear cell invasive. In model 2, the reference group includes all women who did not use genital powders (non-users and non-genital users combined).

†ORs were estimated using conditional logistic regression conditioned on 5yr age groups and adjusted for age (continuous), oral contraceptive duration (never use, <2yrs, 2–<5yrs, 5–<10yrs, >=10yrs), parity (0,1,2,3,4+ children), tubal ligation history (no, yes), BMI (quartiles), race/ethnicity (non-Hispanic white, Hispanic white, black, Asian, other).

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Table 5

Association between estimated lifetime applications of genital powder and risk of ovarian cancer (borderline and invasive combined)

Lifetime number of applications*		All Cases (N= 7,587)		Non-mucinous cases (N= 6,361)	
	Controls (%)	Cases (%)	OR† (95 % CI)	Cases (%)	OR† (95 % CI)
Never users	6175 (76)	5384 (71)	1.00	4472 (70)	1.00
Quartile 1	509 (6)	534 (7)	1.14 (1.00–1.31)	467 (7)	1.18 (1.02–1.36)
Quartile 2	512 (6)	541 (7)	1.23 (1.08–1.41)	456 (7)	1.22 (1.06–1.41)
Quartile 3	497 (6)	542 (7)	1.22 (1.07–1.40)	457 (7)	1.22 (1.06–1.40)
Quartile 4	486 (6)	586 (8)	1.32 (1.16–1.52)	509 (8)	1.37 (1.19–1.58)
p-trend‡			0.17		0.17

*Age specific 25th, 50th and 75th percentile cutpoints are 612, 1,872, and 5,400 for participants < 40 years old; 612, 2,160, and 7,200 for 41–50 years; 720, 3,600, and 10,800 for 51–60 years; 1,440, 5,760, and 14,440 for 61–70; 840, 7,200, and 18,000 for > 70 years.

†ORs were estimated using conditional logistic regression conditioned on 5yr age groups and adjusted for age (continuous), oral contraceptive duration (never use, <2yrs, 2–<5yrs, 5–<10yrs, >=10yrs), parity (0,1,2,3,4+ children), tubal ligation history (no, yes), BMI (quartiles), race/ethnicity (non-Hispanic white, Hispanic white, black, Asian, other).

‡Trend excludes never users.

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